DOCTORAL THESIS

Different shoot and root responses to low phosphorus availability in Japanese cultivars of maize and soybean

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DEDICATION

This dissertation is dedicated to my husband, Mr. Chirath Prabodha Karunarathne, who has been a constant pillar of support and encouragement during the challenges of graduate school and life endeavors. I'm truly thankful for having you in my life. To my parents, Mr. Nihal Karunarathne and Mrs. Ramya Chandani, who raised me to be a kind and strong person. To my loving two sisters, Mrs. Wathsala Karunarathe and Mrs. Duminda Karunarathne, who always loved me unconditionally and inspired me to achieve my goals.

"Never to forget where you came from and always praise the bridges that carried us over."

-Fannie Lou Hamer

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ABBREVIATIONS

Al	Aluminum
AMF	Arbuscular mycorrhizal fungi
ACP	Acid phosphatase
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
Ca	Calcium
CCC	Cubic clustering criterion
DNA	Deoxyribonucleic acid
Fe	Iron
LMA	Leaf mass ratio
MANOVA	Multivariate analysis of variance
N	Nitrogen
NADP	Nicotinamide adenine dinucleotide phosphate
NARO	National Agriculture and Food Research Organization
NP	Nitrophenol
P	Phosphorus
Pi	Inorganic P
PCA	Principal component analysis
PAE	Phosphorus acquisition efficiency
PAP	Purple acid phosphatase
PPUE	Photosynthetic phosphorus use efficiency
PUE	Phosphorus use efficiency
QTL	Quantitative trait loci
RDW	Root dry weight
RNA	Ribonucleic acid
RRS	Root-to-shoot ratio
RSA	Root system architecture

SDW	Shoot dry weight
SRL	Specific root length
TIF	Tag image file
TRL	Total root length
WHC	Water holding capacity

CHAPTER 1

GENERAL INTRODUCTION

1.1. Phosphorus in soil

As a major plant macronutrient, phosphorus (P) often limits plant growth due to its strong sorption and low mobility in most soils (Schachtman et al., 1998). P is generally derived from the weathering of parent rock material. Soil P is found in two forms, called organic and inorganic P. Plants take up phosphates dissolved in soil solution, mainly $H_2PO_4^-$ and HPO_4^{2-}. The soil anion exchange complex can also adsorb phosphate ions. The phosphates dissolved in the soil solution and those phosphates weakly adsorbed by the exchange complex are available for plants and microorganisms (Bueis et al., 2019). Part of the phosphates, more strongly retained by the exchange complex, can become available in the short term (Bueis et al., 2019). These fractions are called labile forms of P (Yang and Post, 2011). The other P forms are included in primary Ca minerals with low solubility. The most highly recalcitrant forms of phosphorus are the stable forms, which are also in the form of Ca minerals, and the residual forms (Turrión et al., 2000). They are mainly organic forms of P associated with clays and Fe and Al oxides (Zamuner et al., 2008). The proportion of organic P ranges from 20 to 80% of total P, mainly as phytate (Richardson, 1994).

Phosphorus deficiency severely impacts crop yield. Regular application of P-fertilizers can overcome the phosphorus limitation in soil, but such phosphorus is a non-renewable resource (Vaccari, 2009). The most currently known rock phosphate reserves are in a few places: Morocco, followed by the USA and China (Cordell et al., 2009). This unequal global distribution of rock phosphate reserves will be a potential problem in the future and will require policies to regulate access to P reserves (Heuer et al., 2017). On a global scale, large imbalances in the rates of application of P fertilizer exist, with adequate or excess application in Western countries and some Asian countries (e.g., China, Japan and Korea) and with an increasing P deficit in many Asian, African and South American countries (MacDonald et al., 2011). On a global scale, about 50% of agricultural soils are deficient in P (Lynch, 2011). Therefore, regular or excessive P-fertilizer application is not considered viable for improving agricultural productivity. Alternative approaches are required, such as developing crops with greater phosphorus efficiency, defined as the ability to grow and yield in soils with reduced phosphorus availability. It would substantially improve food security while enhancing the sustainability of agriculture.

Developing P-efficient cultivars that produce high yields with reduced P-fertilizer inputs is essential for depleting global P resources and minimizing environmental problems. Plants can improve P efficiency by enhancing P acquisition efficiency (PAE) or P utilization efficiency (PUE). PAE is the ability of plants to take up P from soils, and PUE is the capacity of plants to use acquired P to produce biomass or yield (Dissanayaka et al., 2018).

1.2. General responses of plants to P deficiency

Plants have evolved morphological, physiological, and biochemical responses (Zhang et al., 2014) to cope with P deficiency. When plants experience low P availability in the soil, the root system can undergo a range of adaptive responses, including changes in root morphology and architecture, exudation of organic acids and phosphatases into the rhizosphere soil, enhancing expression of high-affinity inorganic P (Pi) transporters in roots and association of arbuscular mycorrhizal fungi (AMF). The most typical responses are the inhibition of photosynthesis and an increase in root-to-shoot ratio (RRS). Under low P availability, shoot growth is retarded due to increased carbon allocation from shoots to roots (Hermans et al., 2006). Other P deficiency responses include the accumulation of anthocyanins (He et al., 2021) and efficient utilization of acquired phosphorus through efficient allocation and mobilization of P within the plant (van de Wiel et al., 2016). However, these morphological and physiological responses to P deficiency are species- and genotype-specific (Liu, 2021).

1.3. Phosphorus acquisition efficiency: strategies for efficient uptake of soil P

1.3.1. Root system architecture (RSA)

Roots have essential functions as a conduit for water and nutrient uptake, and they are targeted for manipulation to improve crop productivity on soils with poor nutrition. Root architecture is the spatial configuration of a root system in the soil, which is vital for plant P acquisition (Lynch, 1995). The root system architecture is highly plastic in its developmental responses to P deficiency. Previous studies have shown that genotypic adaptations to P deficiency cause changes in root architecture that facilitate P acquisition (Chiou and Lin, 2011; Hermans et al., 2006; Péret et al., 2011).

1.3.1.1. Increase in root-to-shoot ratio

Preferred biomass partitioning towards the root is one of the essential adaptive mechanisms of plants under P deficiency (Hermans et al., 2006; Mollier and Pellerin, 1999) because plants allocate more assimilates towards roots, directly involved in nutrient acquisition. Mollier and Pellerin (1999) reported that the root-to-shoot ratio of maize (*Zea mays*) significantly increased in P-deficient conditions compared to sufficient conditions. Further, an increase in root-to-shoot ratio in P0 (No phosphorus application) was found in wheat (Teng et al., 2013) and maize (Deng et al., 2014) compared to high P treatments.

1.3.1.2. Topsoil foraging

Topsoil foraging is strongly associated with P acquisition under low P availability in soils (Zhu et al., 2005b) because Pi availability is usually highest in the upper layers of soil and decreases with depth (Lynch, 2007, 2013). Genetic differences in adaptation to low P availability among genotypes of maize and beans are associated with topsoil foraging (Ho et al., 2005; Zhu et al., 2005a). Architectural traits such as shallower growth of basal roots, enhanced adventitious rooting and greater lateral rooting are associated with enhanced topsoil foraging (Lynch, 2007). Root growth angle is vital in P acquisition and plant yield in low P soil. Zhao et al. (2004) found that the cultivated bush soybean had a shallow RSA and low P efficiency, the wild climbing soybean had a deep RSA and low P efficiency, while the semi-wild soybean had an RSA and P efficiency that were intermediate between those of the cultivated and wild soybean in P deficient soil. The shallow root system for acquiring P through enhanced topsoil foraging has also been observed in maize. In maize, crown roots are the belowground nodal roots primarily distributed in the topsoil (Hoppe et al., 1986) and responsible for nutrient acquisition during vegetative growth and remain important through reproductive development (Lynch, 2013).

Adventitious roots emerge from subterranean hypocotyl (in dicots) or mesocotyl (in monocots) tissue, essential for topsoil exploration. Bean genotypes substantially differ in the extent of adventitious rooting and the regulation of adventitious rooting under low P (Miller et al., 2003; Ochoa et al., 2006). A field study under low P in tropical soil showed that bean genotypes with more adventitious rooting relative to basal root growth had enhanced growth and P uptake. Genetic mapping of adventitious rooting in beans identified several major quantitative trait loci (QTL) that accounted for an impressive

61% of observed phenotypic variation for adventitious rooting in the field under low phosphorus conditions, concluded that adventitious rooting under low phosphorus is a feasible target trait for bean breeding (Ochoa et al., 2006). Adventitious roots may have several benefits for topsoil exploration, and their horizontal growth concentrates foraging activity in the topsoil. Other advantages may relate to the anatomical and morphological differences between adventitious and basal roots. In beans, adventitious roots have greater specific root length (SRL) than other root types.

The third component of root architecture is enhanced lateral rooting, crucial in P acquisition via topsoil foraging. Low P in the rooting zone favors the formation of lateral roots (Lynch, 2007). In maize, substantial genetic variation for lateral rooting exists (Zhu et al., 2005a; Zhu and Lynch, 2004). Genotypes with enhanced or sustained lateral rooting at low phosphorus availability had more excellent phosphorus acquisition and biomass accumulation than genotypes with reduced lateral rooting (Zhu and Lynch, 2004). Lateral root elongation required less biomass and phosphorus investment than the other root types. Genotypes varied in the required phosphorus investment for lateral root elongation and their genetic differences in the SRL and phosphorus concentration of the lateral roots. A large root surface area is achieved by a combination of reduced mean root diameter and elongation of relatively thinner roots (Fitter et al., 2008). Root diameter is critical in exploring soil volume by roots as it determines the volume of soil that the roots can explore (Gahoonia et al., 2006). Plants with a smaller root diameter can explore more soil per unit of root surface area (Fitter et al., 1991) and efficiently uptake P under limiting environments (Gahoonia and Nielsen, 2004).

1.3.1.3. Increased root hair growth

Root hairs from the roots expand the exploratory area around the roots, allowing P to be absorbed from a larger volume of soil (Ma et al., 2021b; Pausch et al., 2016). Root hair formation and growth are regulated by soil P availability (Miguel et al., 2015). Modifications in root hair traits in response to P scarcity include root hair length and density and the location and size of the root hair zone (Brown et al., 2013). Generally, root hairs are not prominent in P-sufficient plants, but when plants experience P deficiency, they increase in length and density (Nestler and Wissuwa, 2016; Zhu et al., 2010). Several major QTL controls genotypic variation in root-hair length and density in maize and beans (Zhu

et al., 2005a), suggesting that this trait could be selected in breeding programs. Genotypic variation in root-hair length and density is essential for phosphorus acquisition regardless of the mycorrhizal status of the plant together with the relatively simple genetic control of these traits and opportunities for direct phenotypic selection, make them attractive criteria for crop breeding programs (Gahoonia and Nielsen, 2004). Several other studies also showed that, relative to P-inefficient maize genotypes, P-efficient maize genotypes often have more extensive root systems with greater root biomass or density (Azevedo et al., 2015; Corrales et al., 2007). In addition to their importance in extending the effective exploratory zone for phosphorus uptake, root hairs may also assist the dispersion of exudates such as carboxylates throughout the rhizosphere, which improves phosphorus bioavailability in many soils (Hinsinger, 2011).

1.3.1.4. Soil exploration at a minimal metabolic cost

The metabolic cost of soil exploration by root systems is substantial, as it can exceed 50% of daily photosynthesis (Lambers et al. 2002). Plants can increase their nutrient use efficiency via cost minimization in low P soils (Lynch and Ho, 2005). Different root types differ in their metabolic cost to the plant. Root costs are considerable under low P stress, which substantially increases root growth relative to shoot growth. A greater RRS means more non-photosynthetic tissue should be sustained, which reduces the overall plant growth rate. Therefore, genotypes with less costly root systems could maintain a larger total root biomass capable of acquiring more soil phosphorus.

Under low P conditions, P-efficient genotypes allocate root biomass to more metabolically efficient root types, such as adventitious and basal (Miller et al., 2003). Further, adventitious and basal roots in beans have greater SRL and lower construction costs than primary roots (Miller et al., 2003). It is advantageous for topsoil exploration because it enables the plant to explore larger soil volumes through reduced metabolic investment in root tissues (Lynch and Ho, 2005). Further, P-efficient genotypes have reduced root respiration at low P for given types of roots due to anatomical adaptations that reduce root costs, such as the formation of root cortical aerenchyma (Galindo-Castañeda et al., 2018; Postma and Lynch, 2011), root cortical senescence (Schneider et al., 2017), root hairs (Lynch, 2011). Adventitious roots may have a greater abundance of

aerenchyma than other root types, which is a mechanism for reducing the metabolic costs of soil exploration.

Additionally, adventitious roots have less lateral branching than basal roots, causing extended root foraging for a given metabolic investment across a larger soil volume (Miller et al., 2003). Root cortical aerenchyma could account for up to 70% and 14% increased growth under P stress in maize and beans, respectively (Postma and Lynch, 2011). Under low P stress, enhanced root hair growth has little metabolic cost to plants.

1.3.2. Root exudates

Many plants have developed specialized root structures in extremely P-impoverished soils with densely clustered lateral roots to release P-mobilizing exudates (Lambers et al., 2006). Several types of root clusters occur in both monocotyledonous and dicotyledonous species. Proteaceae and some species in several other families have bottle brush-like proteoid (cluster) roots, while monocotyledonous families like Restionaceae and Cyperaceae from root clusters termed dauciform and capillaroide roots, respectively. Cluster roots are specialized roots composed of densely spaced tertiary lateral rootlets (Lambers et al., 2013). These cluster-root-bearing plants are widely grown in Southwest Australia and South Africa and adapted to severely P-deficient soils. As Keerthisinghe et al. (1998) reported, acquisition capacity within the cluster root zones is much greater than that of normal roots, confirming the role of cluster roots in P acquisition. Although the formation of cluster roots significantly increases exploratory root area and, thereby, P acquisition, it enhances P acquisition through root exudation mainly rather than P foraging.

1.3.2.1. Organic anions

Plant roots secrete organic acids during the process of acidification. Briefly, Organic anions can compete for sorption sites on soil minerals that might otherwise bind organic and inorganic phosphorus ions and replace P in the sparingly-soluble complexes that form with aluminum (Al), iron (Fe) and calcium (Ca) (Wang and Lambers, 2020). The released inorganic phosphate can be taken up by plant roots directly. The composition of root-secreted organic anions is highly variable and dependent on plant species and cultivars (Badri and Vivanco, 2009). Malate and citrate are the primary organic acids released by roots under P deficiency. Different organic anions have different P-mobilization

capacities in soil; citrate is the most effective in most cases (Jones and Darrah, 1994). White lupin (*Lupinus albus*) is a model crop species that uses root-exuded citrate to cope with P deficiency under hydroponic and soil conditions (Cheng et al., 2011). Recent studies also suggest that oat (*Avena sativa*) root can exhibit a fast exudation rate of citrate under hydroponic conditions and accumulate high concentration of rhizosphere citrate under soil conditions in response to P deficiency (Wang et al., 2016, 2018). However, the increase in the exudation of organic anions under P deficiency was no means to plant species in all studies (Nadeem et al., 2022). As Nadeem et al. (2022) mentioned, a tight correlation between rhizosphere organic anions and plant P acquisition cannot be expected if the plant releases very little organic anions under low P deficiency or root-released organic anions are rapidly sorbed to soil particles or metabolized by soil microorganisms.

In addition, carbon cost is an essential component of adaptation to low P availability, and there will be trade-offs among various strategies. Although plants have evolved various strategies to cope with low P availability, all these strategies require photosynthetic assimilates (Lynch and Ho, 2005; Ryan et al., 2012). Among those strategies, root hair formation improves P acquisition at a minimal carbon cost, while mycorrhizal symbiosis and root exudates increase P acquisition at a significant carbon cost (Lynch et al., 2005; Raven et al., 2018). Gamalero et al. (2003) reported that root exudates account for about 0.2-7% of root dry matter daily. Therefore, there should be a balance between carbon fixation and carbon cost for plants under low P availability, emphasizing that organic anions should be released economically just at certain conditions (e.g., extremely-low plant-available P in the rhizosphere and enough less-available P) and certain growth stages (e.g., high P demand or low internal P concentration) (Wang and Lambers, 2020). Interestingly, a recent study showed a P-efficient soybean genotype (*Glycine max*), which exhibited a higher exudation rate of carboxylates and photosynthetic P use efficiency (Vengavasi and Pandey, 2018). This study further indicates that some P-efficient plants may have evolved strategies to cope with low-P-stimulated large amounts of root exudation (Wang and Lambers, 2020).

1.3.2.2. Phosphatases

Another plant adaptation strategy to low P stress is to increase the accumulation of extracellular acid phosphatase (ACP), which facilitates the scavenging of Pi from organic forms of P. Release of organic forms of P through hydrolysis caused by phosphatase or phytase enzymes. The process of hydrolyzing P from orthophosphoric monoesters depends on soil pH. Generally, the pH optimum for alkaline and acid phosphatase activity is pH 8-10 and 4-7, respectively. Phosphatases and phytases in soil may have a microbial origin (Tarafdar et al., 2001), but roots also exude phosphatases (Tarafdar et al., 2001; Tarafdar and Claassen, 2005), and roots of some species also release significant amounts of phytases (Li et al., 1997a). Phytate can be a major component of the soil organic P pool (Richardson et al., 2011). Most plants cannot access phytate in the rhizosphere except in the presence of phytate hydrolyzing microorganisms (Richardson et al., 2001). Phytases hydrolyze phytate-P, which is not hydrolyzed by most phosphatases. However, a recent study reported that a root-associated purple acid phosphatase in stylo (*Stylosanthes guianensis*) could exhibit high phytase activity and thus facilitate extracellular phytate-P utilization (Liu et al., 2018a). Furthermore, Xiao et al. (2005) summarized that transgenic plants of *Arabidopsis thaliana*, exhibiting enhanced root exudation of extracellular phytase, have greater access to phytate than their wild-type plants.

The expression and exudation of phosphatases by microorganisms and plant roots are regulated by P demand and availability (Maseko and Dakora, 2013). Acid phosphatases can hydrolyze a range of organic P compounds to release Pi for plant uptake (Tarafdar and Claassen, 2005). When plants are P-starved, these enzymes are more abundant in the rhizosphere (Klepper, 1992; Li et al., 1997b; Wasaki et al., 2003).

1.3.2.3. Protons

Proton release is mainly due to the plant's nitrogen (N) nutrition, as related to the balance of cations over anions taken up (Hinsinger et al., 2003). N can be positively charged and favors proton release associated with rhizosphere acidification, or negatively charged (NO_3-N) and favors hydroxyl release associated with alkalization, or uncharged in the case of legumes reliant on N_2 fixation (Hinsinger et al., 2003; Tang et al., 2004). Legumes absorb more cations than anions through N_2 fixation, thus acidifying rhizosphere soil. Increased rhizosphere acidification in response to P deficiency has been reported for

many species, including nitrate-fed legumes (Hinsinger et al., 2003; Neumann and Römheld, 1999). Acidification of the rhizosphere is associated with organic acid exudation and the release of protons from white lupin and chickpea (*Cicer arietinum*) roots, according to the reports of (Neumann and Römheld, 1999; Sas et al., 2001). Sas et al. (2001) have shown that the extrusion of protons and organic acids in white lupin was highly dependent upon P supply. Similarly, Yan et al. (2002) reported substantially enhanced proton release from cluster roots of P-deficient white lupin. Tomato (*Lycopersicon esculentum*) showed an increase in the net release of protons from the roots of P-starved tomato while decreasing organic acid exudation under P deficiency (Neumann and Römheld, 1999).

1.3.3. Soil bacteria and fungi

Various bacteria and fungi species can solubilize inorganic P and/ or mineralize organic P, releasing bioavailable P that plants can readily take up (Singh et al., 2022). Bacterial strains *Pseudomonas*, *Bacillus* and *Rhizobium* are among the most potent P-solubilizing microorganisms (Rodríguez and Fraga, 1999). Immobilized inorganic P can be released through microbial-secreted organic acids, gluconic and citric acid (Singh et al., 2022), and anions from these organic acids chelate cations that otherwise precipitate P-containing anions. The secreted organic acid can cause acidification of the soil environment, releasing P from soluble hydrogen and dihydrogen phosphates (Sharma et al., 2013). Proton extrusion through H^+-Adenosine triphosphatase (ATPase) via transporter-assisted cation exchange across the microbial membranes or in the form of inorganic acids is also possible, which causes acidification (Alori et al., 2017). Microbial mineralization of organic P is mainly catalyzed by non-specific acid phosphatases that dephosphorylate organic molecules and phytases that specifically hydrolyze phytate, the most abundant form of organic P in soil (Lim et al., 2007). Fungi may be considered more effective tools than bacteria in promoting plant P acquisition and can transmit phosphate to plants through symbiotic relationships (Sharma et al., 2013). Hence, associations of plants with soil microbes, such as arbuscular mycorrhizal fungi (AMF), ectomycorrhizal fungi, and P-solubilizing bacteria, can significantly enhance the P-acquisition efficiency of crops (Kafle et al., 2019; Wang et al., 2013). The genotypes of different plant species have different capacities to modify their rhizosphere microbial communities (Turner et al., 2013).

1.3.3.1. Formation of AMF association

The formation of arbuscular mycorrhizal association is an important P-acquisition strategy. Over 70% of terrestrial vascular plant species can form AMF associations (Turner et al., 2013). More recent mycorrhizal associations include ectomycorrhizas and orchidaceous mycorrhizas (Brundrett, 2002). Ectomycorrhizas enhance phosphorus acquisition via mobilization of sparingly soluble phosphorus, whereas both ectomycorrhizas and arbuscular mycorrhizas common in many annuals and hardwood species enhance phosphorus acquisition by increasing the volume of soil explored through the mycorrhizal hyphae (Lynch, 2007). The potential soil exploration efficiency of AMF hyphae is estimated to be six times greater than fine roots alone (McCormack and Iversen, 2019).

Moreover, AMF may also mobilize P from various P pools, for example, organic and sorbed P, and possibly apatite (Andrino et al., 2019, 2021). Possible mechanisms include the release of phosphatases (Zeng et al., 2018) or the promoting P-mobilizing bacteria that release carboxylates and phosphatases (Jiang et al., 2021; Zhang et al., 2016). These carboxylates may function similarly to those released from plants, but the amounts released are minimal compared to those released by roots (Ding et al., 2021). Moreover, maize-associated mycorrhizal fungi release fructose and trigger P-solubilizing bacteria, which mineralize phytate (Zhang et al., 2018).

AMF consume the host plant's photosynthates (up to 20% of daily photosynthate production) (Bago et al., 2000; Parihar et al., 2020). Plants transfer photoassimilates via arbuscular fungal structures in the root cortex to external hyphae in soil. The carbon cost of mycorrhizal symbioses can be a significant component of the metabolic cost of phosphorus acquisition. The more significant metabolic burden of mycorrhizal roots may contribute to the non-beneficial or even parasitic role that mycorrhizal fungi play in agroecosystems (Ryan and Graham, 2002).

1.4. Phosphorus utilization efficiency: strategies for efficient use of acquired P

1.4.1. P uptake and translocation

Pi is generally required at a higher concentration inside the plant cell (5-10 mM). Nevertheless, Pi is commonly present in the soil solution at low concentrations (less than 2 μM) (Raghothama and Karthikeyan, 2005). Thus, Pi can be actively taken up against a concentration gradient by Pi transporter proteins in the plasma membrane of epidermal root

cells. Plants evolve into two Pi uptake systems: high-affinity and low-affinity Pi uptake systems. High-affinity Pi transporters in plants are encoded by PHOSPHATE TRANSPORTER (PHT) genes phylogenetically classified into five families, PHT1-5 (Wang et al., 2017). Phosphate Transporter1 (PHT1) are high-affinity Pi transporters, which play pivotal roles in phosphorus uptake from soils under phosphorus-limited conditions (López-Arredondo et al., 2014). PHT1 genes are induced in Arabidopsis under Pi-limiting conditions (Raghothama and Karthikeyan, 2005). PHT1 transporters have been characterized in other crops such as maize, rice (*Oryza sativa*), wheat (*Triticum aestivum*), soybean, tomato, and barley (*Hordeum vulgare*) (Wang et al., 2017). Moreover, maize ZmPHT1 genes are induced by Pi starvation and mediate the symbiotic association with AMF (Ojeda-Rivera et al., 2022). Besides the direct uptake of phosphorus from soils, plants often acquire additional phosphorus from deep soils with the help of AMF fungal hyphae. During plant-AMF association, three types of PHT1 genes are involved in P absorption, i.e., fungal PHT1 genes, plant PHT1 genes and plant PHT1 genes induced by AMF (Javot et al., 2007). The fungi first assimilate phosphorus by fungal PHT1 transporters and then transfer phosphorus to the plant via AMF-inducible plant PHT1 transporters (Javot et al., 2007; Walder et al., 2015).

Proteins belonging to the PHT1-5 families of Pi transporters play additional roles in maintaining Pi homeostasis by facilitating Pi uptake or remobilizing internal Pi among different tissues or organelles. It is imperative because most of the Pi in the cell is stored in the vacuole, while only 1-5% is present in the cytoplasm (Wang et al., 2017). Vacuole P is used to buffer the Pi demands of the cytoplasm. Once Pi is taken up by the root cells, root-to-shoot translocation of Pi is enabled by PHOSPHATE1 (PHO1), AtPHO1 in Arabidopsis, a protein involved in loading Pi into the xylem (Hamburger et al., 2002). Orthologs for PHO1 have been identified and characterized to have similar functions in soybean, rice, and maize (Salazar-Vidal et al., 2016; Wang et al., 2019b).

1.4.2. P scavenging and remobilization

Besides the induction of high-affinity Pi transport and translocation in response to low P, the activation of P remobilization, scavenging and recycling mechanisms is essential to enhance the P utilization efficiency of acquired P in the plant cell. Plants recycle Pi from the hydrolysis of phospholipids, an essential component in cell membranes, to increase

internal Pi availability (Plaxton and Tran, 2011). Galactolipids and sulfolipids, rather than phospholipids, are the major non-Pi lipids in the thylakoid membrane. Therefore, non-Pi lipids like galactolipids and sulfolipids replace phospholipids to maintain the functionality and structure of the plasma membrane in response to P deficiency. Hence, plants can gain the PUE through membrane lipid remodeling under low P stress to reduce P investment in the phospholipid pool. Interestingly, plants belonging to the Proteaceae family predominantly use galactolipids and sulfolipids instead of phospholipids in mature leaves, whereas expanding leaves contain relatively large amounts of phospholipids allowing them to maintain high photosynthetic P use efficiency (Kuppusamy et al., 2014). Moreover, in soybeans, amounts of phospholipids in young leaves grown under P-limited conditions are almost similar to those under P-sufficient conditions, whereas P-deficient mature leaves replaced their phospholipids with non-Pi lipids (Okazaki et al., 2017).

Phospholipid hydrolysis and Pi-recycling from nucleic acids contribute to buffering the cytosolic Pi pools when Pi is limited (Plaxton and Tran, 2011; Jeong et al., 2017). Maintaining adequate cytosolic Pi levels is crucial because low P negatively impacts photosynthesis, eventually inhibiting plant growth and development. Pi scavenging in plants is promoted through the upregulation of genes encoding acid phosphatases, which hydrolyze Pi from organic Pi-esters present in a wide variety of organic compounds (e.g., nucleic acids, ATP, 3-phosphoglycerate, and various hexose-Pi compounds) in P-deprived plants and senescing leaves (Plaxton and Tran, 2011; Tran et al., 2010). The nucleic acid pool is typically a plant's largest organic P pool, contributing approximately 40-60% of the P found in the combined organic P pool. A significant proportion of the nucleic acid pool generally contains RNA (85%), where most RNA is ribosomal RNA (rRNA). As rRNA is a major sink for P, its degradation would yield considerable amounts of Pi (Veneklaas et al., 2012). Intracellular and secreted (cell wall and apoplast) purple acid phosphatases (PAPs) and ribonucleases play a crucial role in hydrolyzing Pi in senescing leaves for translocation to growing organs where P demand is high (Dissanayaka et al., 2021).

Many studies have focused on the role of plant purple acid phosphatases in Pi scavenging and recycling during Pi starvation. Vacuolar or other intercellular PAPs are expressed in temporal and tissue-specific fashion to mobilize Pi from storage organelles or senescent leaves (Gao et al., 2017; Li et al., 2002). In Arabidopsis, AtPAP26 is the

predominantly secreted, intracellular (vacuole) phosphatase under low P stress (Li et al., 2002; Tran et al., 2010). Putative PAPs have been reported in maize (González-Muñoz et al., 2015), soybean (Li et al., 2012), and rice (Zhang et al., 2011). Enhanced levels of PAPs remain an exciting prospect for boosting P efficiency in crops.

1.5. Maize and soybeans

Wheat, maize, and rice are the leading staple cereals. Maize, a monocot, is a multi-purpose crop compared to wheat and rice, and it is used as a human diet, livestock feed, industrial and energy crop. Maize plays a diverse and dynamic role in the global agricultural food system. On the other hand, soybean, a dicot, is an economically important legume in the world as the richest and cheapest source of protein. It is a staple diet of humans and animals in numerous parts of the world. Cereal-legume intercropping is recognized as the most popular agricultural practice in many developing countries in the world. Numerous studies revealed that maize and soybean are best partners under intercropping systems because both have complementary characteristics. They are the N-consuming C_4 and N-fixing C_3 crops. Maize and soybean have large, cylindrical and small round leaf shapes, respectively, suitable for efficient light utilization on the same land (Iqbal et al., 2019). In such scenarios, root architecture is essential in determining root systems' spatial competition and complementarity (van Noordwijk et al., 1996).

1.6.The exploitation of genotypic variation for low P tolerance

Maize and soybean are two major food crops and can be grown in a wider variety of soil and climatic conditions. The scarcity of available P in soil solutions severely limits the growth and yield of most crops. However, applying large amounts of chemical P fertilizers is not a viable solution because rock P deposits, the only source of P fertilizers, are non-renewable. Therefore, a potential global P crisis has been extensively debated during the last several years (Cordell and White, 2014). Genetic diversity among species and genotypes provides an opportunity to improve low P tolerance. Therefore, exploiting genotypic variation in crop responses to low P stress is a promising tool for breeding P-efficient genotypes.

Researchers have worked to understand how plants adapt to low P stress and the mechanisms that increase P uptake, transport, and utilization in the past few years.

Therefore, improvements in crop nutrition to maximize PAE and PUE are urgently needed to secure food production while opening pathways for sustainable agriculture. The uptake of available P occurs through the action of several Pi transporters and is greatly influenced by the root explorative and scavenging capacity, determined mainly by the RSA. As discussed above, modifications to the RSA can be crucial for a plant to adapt to low P stress because roots are the entry points of P to the plant. The QTLs that associate root traits of P-efficient genotypes, such as lateral root branching, adventitious root formation, and root hair growth, have been identified in rice, common bean, and maize (Liao et al., 2004; Liu et al., 2018b; Zhu et al., 2005a).

However, broader germplasm must have a greater potential for genotypic variation. Evaluation of P-efficient germplasm among existing Japanese landraces is of interest because landraces are well adapted to low P environments and may possess traits not common in elite germplasm. Furthermore, no reports appear to be available for the low P tolerance of Japanese landraces of maize and soybean.

1.7. Aims of the study

The study aimed to evaluate

(1) genotypic variability of Japanese core collections of maize and soybean in response to low P availability,

(2) different shoot and root responses of selected Japanese cultivars of maize and soybean,

(3) acid phosphatase (ACP) activity and rhizosphere acidification of selected Japanese cultivars of maize and soybean and

(4) to compare shoot and root responses to low P availability between two species.

CHAPTER 2

SCREENING JAPANESE CORE COLLECTIONS OF SOYBEAN AND MAIZE UNDER LOW P CONDITIONS

2.1. Introduction

Low phosphorus (P) availability in agricultural soils severely impacts crop productivity worldwide. P is the second most growth-limiting macronutrient, determining the crucial roles in plant systems (Hawkesford et al., 2012; Hou et al., 2020). Application of rock phosphate fertilizers is inefficient as up to 80% of all fertilizers are quickly modified, immobilized or transformed into insoluble organic P derivatives and become unavailable to plants (Balaban et al., 2017). Consequently, P deficiency is a major yield-limiting factor in acidic and calcareous soils, where P retention is high (Hinsinger, 2011).

Higher plants have adapted different mechanisms to enhance P acquisition efficiency (PAE) and/ or P use efficiency (PUE) to withstand P-deficient conditions. PAE refers to the ability of crop genotypes to take up more P from soils, and PUE is the ability to produce more biomass or yield using the acquired P (Wang et al., 2010a). Strategies related to PAE and PUE are equally essential to improve the P efficiency of crops. High P acquisition strategies include root foraging and root mining strategies. Root foraging strategies enable plants to take more P by exploring large volumes of soil. Root mining strategies like root exudates, organic anions, and phosphatases enhance P acquisition through desorption and mineralizing sparingly available P and organic P pools. These mechanisms are equally crucial for acquiring more P into plants under low P conditions. 'Improved internal P utilization efficiency' can be achieved through optimal distribution and redistribution of P to harvestable plant parts to allow maximum growth and biomass allocation under low P (Richardson et al., 2011). Plant species and their cultivars widely differ in P efficiency because of differences in one or more of these mechanisms (Aziz et al., 2014). Therefore, exploiting the genetic variability of crop genotypes under low P conditions and developing P-efficient crop genotypes are crucial to keep the momentum of sustainable agriculture.

Landraces and naturally inbred traditional cultivars have been cultivated under P-deficient soils for extended periods. There may be plenty of chances to have P-efficient crop germplasm in landraces (Yao et al., 2007). NARO (National Agriculture and Food Research Organization) gene bank of Japan has developed a core collection of Japanese landraces of several crop species for research purposes. A core collection is a limited set of accessions representing the genetic diversity of a crop species and its wild relatives

with minimum repetitiveness (Frankel, 1984). Japanese core collections of maize and soybean have yet to be tested for low P stress.

Soybean (*Glycine max* L.) is an economically important legume crop belonging to the family Fabaceae. It is one of the richest and cheapest protein sources for humans and animals. Soybean is abundantly cultivated in tropical, subtropical, and temperate regions, where the soils are often deficient in P due to intensive soil erosion, weathering and P retention in the soils (Hanway and Olson, 1980). Whereas maize (*Zea mays* L.) is a globally important cereal crop belonging to Poaceae. It is grown worldwide for food, feed, and fuel. A sufficient level of P in soil solution is pivotal for the optimum growth and yield of maize. In Japan, the demand for soybeans and maize remains robust. Japan is heading to lower rice production while moving to promote the cultivation of soybean and forage crops like wheat and maize. At the same time, these crop species are essential in diversified cropping systems like intercropping or rotations. Accordingly, genotypic strategies could be combined with agronomic strategies to enhance P efficiency jointly (Cong et al., 2020). In diversified cropping systems like intercropping, P efficiency can be effectively enhanced by including genotypes characterized by high P acquisition or P mobilizing.

As mentioned in previous studies, plant growth and development were severely retarded due to P deficiency. Nevertheless, some plants or cultivars will die; meanwhile, some plants or cultivars can still grow generally under low P conditions. It implies differences in P acquisition and utilization in different plants or cultivars (Meng et al., 2014). Therefore, screening large germplasm under low P conditions is the way to identify P-efficient cultivars, known as selection. Further, screening and selecting for P efficiency in the early stages of crop growth and development can reduce the duration of the test cycle and workload (Wang et al., 2021). Therefore, assessing crop species and their genetic variability, understanding their genetic potential, and incorporating them in breeding programs is crucial for improving the low P-tolerant crop genotypes.

2.2. Objectives

The study aimed to screen both Japanese core collections of soybean and maize and examine whether some cultivars of both species exhibit low P tolerance.

2.3. Materials and methods

The study was compromised of two separate screening experiments: soybean and maize under hydroponic conditions. They were screened under two different P concentrations. At first, we screened the Japanese soybean core collection under low P (50 µM) for 30 days during November – December 2021 and then the maize core collection under low P (2 µM) again for 30 days during March – April 2022. Cultivars were evaluated for shoot and root growth at the harvest.

2.3.1. Cultivars

The soybean core collection of Japanese landraces contains 94 cultivars, and the Japanese maize core collection (year 2021 set) contains 86 cultivars (Kaga et al., 2011). The collections were obtained from the Research Center of Genetic Resources, NARO, Tsukuba, Japan (NARO Genebank Project, Japan). The complete lists of landraces examined in this study are shown in Tables 2.1 and 2.2.

2.3.2. Plant growth conditions of soybean in hydroponic culture

Both screening experiments were carried out in the glasshouse at Hiroshima University, Japan. Seeds were germinated in moist vermiculite-filled seedling trays. After germination, seedlings were transferred to 180 L containers (1.06 × 0.73 × 0.30 m^3). The containers were filled with a half-strength modified Hoagland solution with low P (50 µM), as mentioned in Zhou et al. (2016). The composition of the nutrient solution was as follows: 0.75 mM K_2SO_4, 2 mM $Ca(NO_3)_2$, 0.65 mM $MgSO_4$, 0.1 mM KCl, 0.05 mM KH_2PO_4, 0.1 mM Fe(III)-EDTA, 10 µM H_3BO_3, 1 µM $MnCl_2$, 0.1 µM $CuSO_4$, 1 µM $ZnSO_4$, 0.5 µM $(NH_4)_6Mo_7O_{24}$. The solution was well aerated and renewed every five days, and pH was maintained daily at 5.4-5.5 by adding 1M HCl or 1M NaOH. Each cultivar had four replicates. Plants were harvested after 30 days of transplanting.

2.3.3. Plant growth conditions of maize in hydroponic culture

Seeds were germinated in moist vermiculite-filled seedling trays. After germination, seedlings were transferred to 180 L containers (1.06 × 0.73 × 0.30 m^3). The containers were filled with nutrient solution containing low P (1 µM), as Gong et al. (2011) mentioned. Two weeks after screening, the P concentration of the nutrient solution was increased to 2 µM. The composition of the nutrient solution was as follows: 0.75 mM

K_2SO_4, 2 mM $Ca(NO_3)_2$, 0.65 mM $MgSO_4$, 0.1 mM KCl, 0.001 mM KH_2PO_4, 0.1 mM Fe(III)-EDTA, 1 µM H_3BO_3, 1 µM $MnCl_2$, 0.1 µM $CuSO_4$, 1 µM $ZnSO_4$, 0.5 µM $(NH_4)_6Mo_7O_{24}$. The solution was well aerated and renewed every five days, and pH was maintained daily at 6.0 using 1M HCl or 1M NaOH. Each cultivar had four replicates. Plants were harvested after 30 days of transplanting.

2.3.4. Plant analysis

At harvest, the shoot and roots were separated. The root systems were thoroughly washed with distilled water and stored in distilled water-filled plastic containers at 4 ºC until root scanning. Shoots were oven-dried at 80 ºC for three days. The roots were cut into segments and floated in a transparent acrylic tray for digital images using an image scanner and a positive film transparency unit (Epson Gt-X970, Seiko Epson Corp. Nagano, Japan). 8-bit grayscale images were taken at a 400-dpi resolution. The root images were saved in TIF format. Using the free software ImageJ (https://imagej.nih.gov/ij/), the TRLs of the scanned images were calculated according to Tajima and Kato (2013).

After root scanning, root samples were oven-dried at 80 ºC for three days. Shoot dry weight (SDW), root dry weight (RDW), root-to-shoot ratio (RRS), and specific root length (SRL) were evaluated. SRL is the ratio of TRL to RDW. All dried shoot samples were ground. Ground plant samples (200 mg) were digested for P determination using the HNO_3 and H_2O_2 digestion method described in Wheal et al. (2011). The P concentrations of extracts were quantified spectrophotometrically (UV-1800, Shimadzu Corporation, Kyoto, Japan) using the phosphomolybdate-blue method (Murphy and Riley, 1962). P contents in the shoot were calculated by multiplying the shoot dry weight with the P concentration in the shoot. PUE (dry weight per unit P uptake) was calculated as shoot DW divided by shoot P content (Moll et al., 1982).

2.3.5. Statistical analysis

Cluster analysis was carried out using Ward's method of hierarchical clustering using SAS 9.4 software, SAS Institute Inc, Cary, NC, USA. The classification was based on the shoot and root growth: SDW, RDW, TRL, RRS and SRL under low P stress imposed in hydroponic conditions. Cluster means were calculated by taking the mean value of each variable in each Cluster. The number of clusters was determined based on the pseudo-*F*

statistic, cubic clustering criterion (CCC) and pseudo t^2 statistic graphs analyzed by SAS 9.4 software, SAS Institute Inc, Cary, NC, USA. Multivariate analysis of variance (MANOVA) was performed to test the differences across the clusters.

Table 2.1 Summary of the Japanese soybean core collection

Experimental ID	gene bank ID	Name	Origin
1	GmJMC002	WASE KURO DAIZU	Japan (Kumamoto)
2	GmJMC003	NATSU KURAKAKE	Japan (Kumamoto)
3	GmJMC004	KITAJIRO	Japan (Chiba)
4	GmJMC005	WASEOUSODE (SHIKAOI ITOH)	Japan (Hokkaido)
5	GmJMC007	TOKACHI NAGAHA	Japan (Hokkaido)
6	GmJMC008	KANAGAWA WASE	Japan (Kumamoto)
7	GmJMC009	SHIZUNAIDAIZU	Japan (Hokkaido)
8	GmJMC013	CHIZUKA IBARAKI 1	Japan (Ibaraki)
9	GmJMC016	JUKKOKU	Japan (Saitama)
10	GmJMC021	OOYACHI 2	Japan (Hokkaido)
11	GmJMC023	KUROGOYOU	Japan (Fukushima)
12	GmJMC025	ENREI	Japan (Nagano)
13	GmJMC026	ONI HADAKA	Japan (Tochigi)
14	GmJMC028	KOITO	Japan (Chiba)
15	GmJMC030	KURODAIZU (AO HIGUU CHUU)	Japan (Okinawa)
16	GmJMC031	SHIRO MITSU MAME	Japan (Nagano)
17	GmJMC032	NATTOU KOTSUBU	Japan (Ibaraki)
18	GmJMC033	BANSEI HIKARIKURO	Japan (Hokkaido)
19	GmJMC034	MIYAGI SHIROME	Japan (Miyagi)
20	GmJMC037	YAKUMO MEAKA	Japan (Hokkaido)
21	GmJMC039	NATTOUMAME	Japan (Nagano)
22	GmJMC040	KOIBUCHIMURA ZAIRAI	Japan (Ibaraki)
23	GmJMC041	DATE CHA MAME	Japan (Miyagi)
24	GmJMC043	TAKIYA	Japan (Yamagata)
25	GmJMC044	SHAKKIN NASHI	Japan (Gunma)
26	GmJMC047	AKITA ANI	Japan (Yamagata)
27	GmJMC049	HIKU ANDA	Japan (Okinawa)
28	GmJMC050	FUKUI SHIRO	Japan (Fukui)
29	GmJMC051	KURODAIZU(GEIHOKU)	Japan (Hiroshima)
30	GmJMC052	KISAYA(NATSU)	Japan (Kagoshima)
31	GmJMC053	ABURA MAME	Japan (Fukushima)
32	GmJMC054	ZAIRAI 51-6	Japan (Aichi)
33	GmJMC055	SAKURAMAME	Japan (Yamagata)
34	GmJMC056	TAMAHOMARE	Japan (Nagano)
35	GmJMC057	SHAKUJOU MAME	Japan (Unspecified)
36	GmJMC058	YAHAGI	Japan (Aichi)
37	GmJMC059	SOKOSHIN	Japan (Niigata)
38	GmJMC060	SHIMO HISAKATA DAIZU	Japan (Nagano)
39	GmJMC061	KOMAME	Japan (Gunma)
40	GmJMC062	AZEMAME	Japan (Tochigi)
41	GmJMC063	AOBAKO	Japan (Yamagata)
42	GmJMC064	MEGURO 1	Japan (Aomori)
43	GmJMC065	OOJIRO	Japan (Gunma)
44	GmJMC067	HOUJAKU	Japan (Nagano)
45	GmJMC068	ZAIRAI 51-2	Japan (Aichi)
46	GmJMC069	CHADAIZU	Japan (Miyagi)
47	GmJMC076	IHHON SANGOU	Japan (Ibaraki)
48	GmJMC077	HITORIMUSUME	Japan (Yamagata)
49	GmJMC078	AKASAYA	Japan (Nara)
50	GmJMC079	KURUMIMAME	Japan (Miyagi)
51	GmJMC080	HIME DAIZU	Japan (Gifu)
52	GmJMC081	AKUDEN SHIRAZU	Japan (Nagano)
53	GmJMC082	AOAKIMAME	Japan (Hyogo)
54	GmJMC085	DAIZU	Japan (Wakayama)
55	GmJMC088	CHUU TEPPOU	Japan (Gifu)
56	GmJMC090	DADACHAMAME	Japan (Yamagata)
57	GmJMC091	KUROTOME	Japan (Miyagi)

Continued

Table 2.1 ***Continued***

Experimental ID	gene bank ID	Name	Origin
58	GmJMC092	KUROHIRA	Japan (Iwate)
59	GmJMC093	ZAI 52-12	Japan (Chiba)
60	GmJMC095	AKASAYA	Japan (Ishikawa)
61	GmJMC096	NAKAHATA ZAIRAI	Japan (Shizuoka)
62	GmJMC097	IPPON SUZUNARI	Japan (Aichi)
63	GmJMC098	AKA DAIZU	Japan (Tokushima)
64	GmJMC099	AMAGI ZAIRAI 90D	Japan (Fukuoka)
65	GmJMC100	KUROMAME	Japan (Saitama)
66	GmJMC101	COL/EHIME/1983/UTSUNOMIYA 22	Japan (Ehime)
67	GmJMC102	KURAKAKE	Japan (Niigata)
68	GmJMC104	DAIZU(SHIRO)	Japan (Nara)
69	GmJMC105	MAETSUE ZAIRAI 90B	Japan (Oita)
70	GmJMC106	HIMESHIRAZU	Japan (Chiba)
71	GmJMC110	COL/TANBA/1989/ODAGAKI 2	Japan (Hyogo)
72	GmJMC111	AMAGI ZAIRAI 90A	Japan (Fukuoka)
73	GmJMC112	FUKUYUTAKA	Japan (Kumamoto)
74	GmJMC114	COL/EHIME/1-2	Japan (Ehime)
75	GmJMC116	SHIRATAMA	Japan (Iwate)
76	GmJMC117	AKISENGOKU	Japan (Kumamoto)
77	GmJMC121	KOSA MAME	Japan (Tochigi)
78	GmJMC126	KOKUBU 7	Japan (Hyogo)
79	GmJMC128	GIN DAIZU	Japan (Okayama)
80	GmJMC130	HITASHIMAME	Japan (Yamagata)
81	GmJMC131	COL/EHIME/1983/UTSUNOMIYA 28	Japan (Ehime)
82	GmJMC133	DAIZU	Japan (Kochi)
83	GmJMC137	COL/EHIME/1983/UTSUNOMIYA 37	Japan (Ehime)
84	GmJMC139	BUNSEI	Japan (Kumamoto)
85	GmJMC145	SHIMOTSURA	Japan (Kumamoto)
86	GmJMC149	MOCHI-DAIZU	Japan (Mie)
87	GmJMC158	KUMAJI 1	Japan (Kumamoto)
88	GmJMC161	ITSUKI ZAIRAI 83H	Japan (Kumamoto)
89	GmJMC167	NANKAN ZAIRAI 83	Japan (Kumamoto)
90	GmJMC172	TSURUSENGOKU	Japan (Chiba)
91	GmJMC177	HAI MAME	Japan (Yamanashi)
92	GmJMC179	SAGA ZAIRAI	Japan (Saga)
93	GmJMC180	KOMUTA	Japan (Kumamoto)
94	GmJMC184	BAN KURO DAIZU	Japan (Kumamoto)

The following cultivars were excluded due to poor germination and replication: GmJMC003, GmJMC023, GmJMC025, GmJMC054, GmJMC061, GmJMC063, GmJMC065, GmJMC067, GmJMC069, GmJMC080, GmJMC081, GmJMC091, GmJMC161.

Table 2.2 Summary of the Japanese maize core collection

Experimental ID	gene bank ID	Name	Origin
1	JMC 01	KANAENO 1	Japan (Ooita)
2	JMC 02	TSUBAKI 1	Japan (Kumamoto)
3	JMC 03	SUGINAZAWA 2	Japan (Shizuoka)
4	JMC 04	OKUZURU WASE	Japan
5	JMC 05	YAYAE B	Japan (Kumamoto)
6	JMC 06	KAWACHI 4	Japan (Miyazaki)
7	JMC 07	HIRANO ZAIRAI HACHIRETSU A	Japan (Yamanashi)
8	JMC 08	SHIMONAKA MURAKAMI	Japan (Ehime)
9	JMC 09	NAKASE 1	Japan (Miyazaki)
10	JMC 10	SHOU TOUMOROKOSHI	Japan
11	JMC 11	YAMAKIBI	Japan (Kouchi)
12	JMC 12	AKABANE 2	Japan (Kumamoto)
13	JMC 13	IIBOSHI 2	Japan (Miyazaki)
14	JMC 14	KAMIGANE 3	Japan (Yamanashi)
15	JMC 15	ASAKABE 5	Japan (Miyazaki)
16	JMC 16	TOUKOUJI 1	Japan (Miyazaki)
17	JMC 17	TORINOSU	Japan (Miyazaki)
18	JMC 18	JURIKI JURIKI 1	Japan (Shizuoka)
19	JMC 19	FUNATSU SHINYA 3	Japan (Yamanashi)
20	JMC 20	KUNI 2	Japan (Gunma)
21	JMC 21	TONE 2	Japan (Gunma)
22	JMC 22	ITAZUMA SUGINAZAWA 1	Japan (Shizuoka)
23	JMC 23	MANBA 2	Japan (Gunma)
24	JMC 24	EHIME SOUGAWAMURA 44	Japan (Ehime)
25	JMC 25	KIBI	Japan (Kouchi)
26	JMC 26	WADA	Japan (Kouchi)
27	JMC 27	YASHIKIKIBI	Japan (Kouchi)
28	JMC 28	KIJIYAMA 33	Japan (Miyazaki)
29	JMC 29	EHIME MIKAWAMURA 54	Japan (Ehime)
30	JMC 30	TOCHINOKI 1	Japan (Miyazaki)
31	JMC 31	YASHIKIKIBI	Japan (Kouchi)
32	JMC 32	KAMINAGOU 1	Japan (Miyazaki)
33	JMC 33	KUMA-MACHI KAMINOJIRI	Japan (Ehime)
34	JMC 34	WADASHU	Japan (Kouchi)
35	JMC 35	OOKAWACHI A	Japan (Miyazaki)
36	JMC 36	MIKADOBARU 4	Japan (Miyazaki)
37	JMC 37	OMUKAE 2	Japan (Miyazaki)
38	JMC 38	KOUSHUU	Japan (Yamanashi)
39	JMC 39	SHIRO TOUKIBI	Japan (Miyazaki)
40	JMC 40	MIZUHIKI 3	Japan (Fukushima)
41	JMC 41	HEINAI 1	Japan (Aomori)
42	JMC 42	TANOHATA 1	Japan (Iwate)
43	JMC 43	NOKATANO 3	Japan (Miyazaki)
44	JMC 44	KIBI	Japan (Kouchi)
45	JMC 45	SUYAMA TSUDOHI 1	Japan (Shizuoka)
46	JMC 46	YAMAMIKE TOUKIBI 7-8	Japan (Miyazaki)
47	JMC 47	NAKABARU 1	Japan (Miyazaki)
48	JMC 48	NISHIHARUCHIKA ZAIRAI	Japan (Nagano)
49	JMC 49	DAIGO MOCHI 2	Japan (Ibaraki)
50	JMC 50	MOCHIKIBI	Japan (Kouchi)
51	JMC 51	TSUKUI YOSHINO	Japan (Kanagawa)
52	JMC 52	SAKANASHI 1	Japan (Kumamoto)
53	JMC 53	YOKOHACHI	Japan (Miyazaki)
54	JMC 54	KOWASE	Japan
55	JMC 55	DOUSHI KAWARABATA	Japan (Yamanashi)
56	JMC 56	TOYOMAKI 4	Japan (Yamanashi)
57	JMC 57	TORIYABE	Japan (Aomori)

Continued

Table 2.2 ***Continued***

Experimental ID	gene bank ID	Name	Origin
58	JMC 58	SHIONOMATA 1	Japan (Fukushima)
59	JMC 59	CHUUAKA TOUMOROKOSHI	Japan
60	JMC 60	ASOO 1	Japan (Ibaraki)
61	JMC 61	JUUGATSUKIBI	Japan (Kouchi)
62	JMC 62	YAGAUCHIKIBI	Japan (Kouchi)
63	JMC 63	HAYAKIBI	Japan (Kouchi)
64	JMC 64	DAIGO MOCHI 3	Japan (Ibaraki)
65	JMC 65	YUNOHANA	Japan (Fukushima)
66	JMC 66	NAKAZATO 2	Japan (Gunma)
67	JMC 67	USHIKU	Japan (Ibaraki)
68	JMC 68	ASOO 3	Japan (Ibaraki)
69	JMC 69	KOUSHUU	Japan (Yamanashi)
70	JMC 70	KAMIKAWA ZAIRAI	Japan (Hokkaido)
71	JMC 71	YUUBARI ZAIRAI A	Japan (Hokkaido)
72	JMC 72	NAKAGAWA ZAIRAI B	Japan (Hokkaido)
73	JMC 73	ASHORO ZAIRAI	Japan (Hokkaido)
74	JMC 74	KAYABE ZAIRAI A	Japan (Hokkaido)
75	JMC 75	KAMEDA ZAIRAI C	Japan (Hokkaido)
76	JMC 76	IWANAI ZAIRAI B	Japan (Hokkaido)
77	JMC 77	HOKKAIDO HACHIGYOU	Japan (Hokkaido)
78	JMC 78	IWANAI ZAIRAI	Japan (Hokkaido)
79	JMC 79	OOE ZAIRAI	Japan (Hokkaido)
80	JMC 80	SAKASHITA TAIKI	Japan (Hokkaido)
81	JMC 81	OBIHIRO NAKAYAMA	Japan (Hokkaido)
82	JMC 82	URAHORO IIYAMA	Japan (Hokkaido)
83	JMC 83	SHIZUNAI 4	Japan (Hokkaido)
84	JMC 84	SAROBETSU	Japan (Hokkaido)
85	JMC 85	NEMURO A	Japan (Hokkaido)
86	JMC 86	BEKKAI KASSHOKURYUU	Japan (Hokkaido)

2.4. Results

2.4.1. Classification of Japanese core collection of soybean

Eighty-one soybean cultivars were classified into four clusters: I, II, III and IV (Figure 2.1). MANOVA results in Table 2.3 revealed that significant differences existed across clusters to the variables SDW, RDW, TRL, RRS and SRL under low P stress. Cluster I and II contained 26 and 19 genotypes, respectively. According to the cluster descriptives, these two clusters differed only through mean TRL (Table 2.4). Cluster III contained 14 genotypes with high SDW, RDW and TRL, indicating low P tolerance (Table 2.4). Cluster IV contained the rest of the genotypes (22) that showed a 50% reduction in SDW compared to Cluster III, indicating they were low P-sensitive (Table 2.4). Cluster IV was characterized by significantly low mean values for SDW, RDW and TRL compared to the rest of the clusters (Table 2.4). Mean RRS and SRL were not significantly different across the four clusters (Table 2.4).

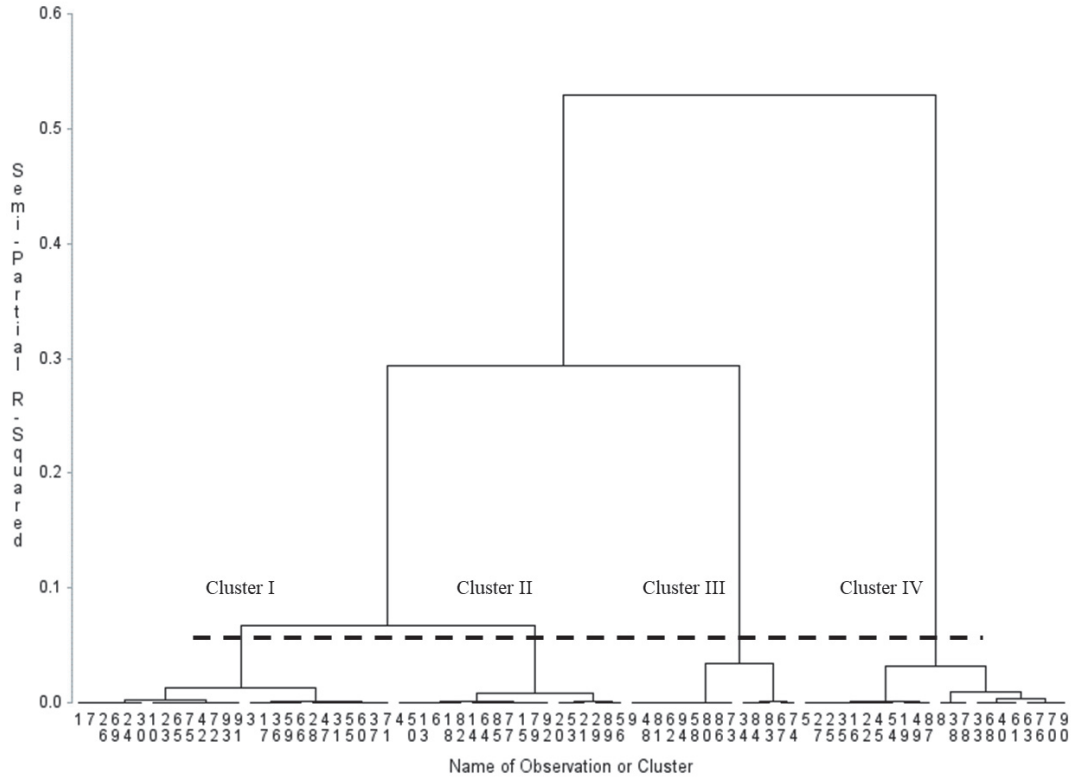

Figure 2.1 Dendrogram of Japanese core collection of soybean.

Table 2.3 MANOVA results for testing differences across clusters of soybean genotypes

Statistics	Value	F	Hypothesis df	Error df	Pr > F
Dependent variables: SDW, RDW, TRL, RRS, and SRL					
Pillai's Trace	1.22	7.18	21.00	219.00	<.001
Wilk's Lambda	0.07	14.96	21.00	204.42	<.001
Hotelling's Trace	9.41	31.23	21.00	209.00	<.001
Roy's Largest Root	8.98	93.64	7.00	73.00	<.001

Degree of freedom (df), probability (Pr). Variables include shoot dry weight (SDW), root dry weight (RDW), total root length (TRL), root-to-shoot ratio (RRS), and specific root length (SRL).

Table 2.4 Descriptive statistics for soybean classification

Cluster	SDW (g)			RDW (g)			TRL (cm)			RRS			SRL (m g^{-1})		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
I	0.90	2.73	1.82b	0.22	0.53	0.37b	3576	4926	4306c	0.13	0.31	0.21a	95.5	194.9	129.4a
II	1.07	3.10	1.98b	0.24	0.61	0.43b	5055	6341	5563b	0.18	0.26	0.22a	100.6	255.5	138.9a
III	2.23	3.18	2.64a	0.43	0.93	0.60a	6605	9040	7501a	0.19	0.29	0.23a	102.3	156.3	126.7a
IV	0.45	2.51	1.23c	0.09	0.39	0.24c	1049	3373	2549d	0.15	0.24	0.19a	94.2	186.4	126.7a

Mean values in a column not followed by a common letter differ significantly ($p < 0.05$). Variables include shoot dry weight (SDW), root dry weight (RDW), total root length (TRL), root-to-shoot ratio (RRS), and specific root length (SRL).

2.4.2. Classification of Japanese core collection of maize

Eighty-six cultivars of maize were classified into four clusters: I, II, III and IV (Figure 2.2). MANOVA results in Table 2.5 revealed that significant differences existed across clusters to the variables SDW, RDW, TRL, RRS and SRL under low P stress. Among the variables tested, only SDW, RDW and TRL impacted the differences across the clusters. Hence, RRS and SRL were not significantly different among the four clusters (Table 2.6). Cluster II contained 6 cultivars with significantly high SDW, RDW and TRL mean values indicating low P tolerance. Cluster I contained 24 genotypes, and Cluster IV, which contained 40 genotypes, was the largest. Cluster I and IV were not significantly different by SDW, but mean RDW and TRL values were significantly higher in Cluster I compared to Cluster IV. Cluster III had significantly lower mean values for SDW, RDW and TRL than the other three clusters. Compared to Cluster II, Cluster III reduced 50% of its mean SDW value under low P conditions, indicating they were sensitive to the low P stress.

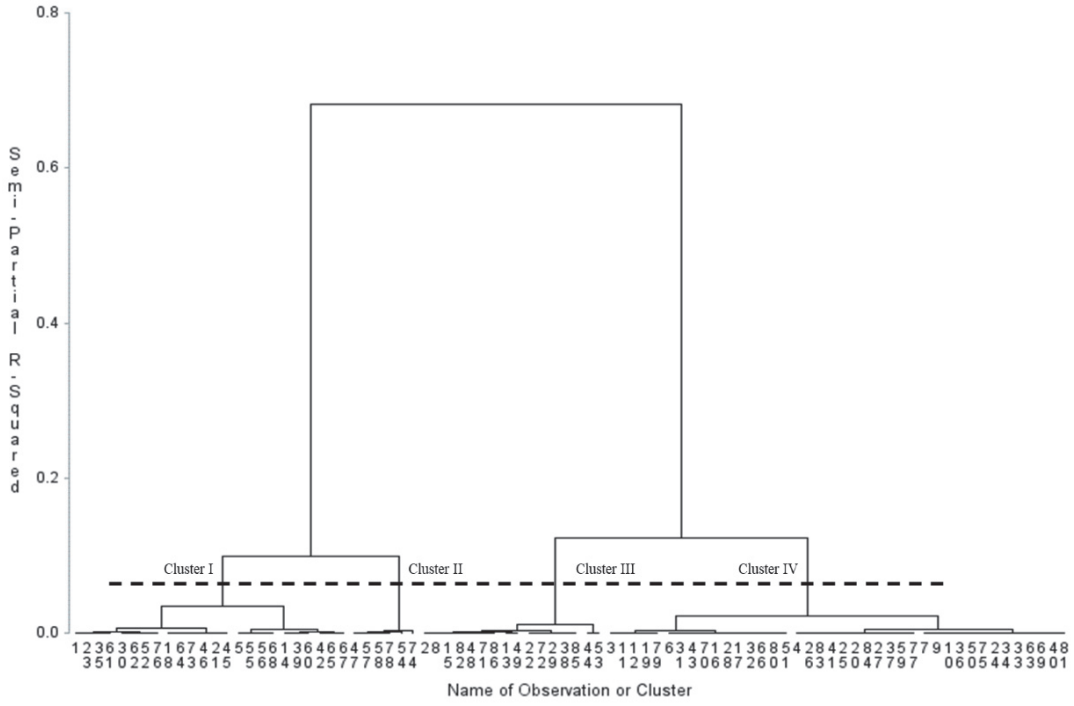

Figure 2.2 Dendrogram of Japanese core collection of maize.

Table 2.5 MANOVA results for testing differences across clusters of maize genotypes

Statistics	Value	F	Hypothesis df	Error df	Pr > F
Dependent variables: SDW, RDW, TRL, RRS, and SRL					
Pillai's Trace	1.08	9.03	15.00	240.00	<.001
Wilk's Lambda	0.08	22.26	15.00	215.72	<.001
Hotelling's Trace	10.20	52.14	15.00	230.00	<.001
Roy's Largest Root	10.00	160.13	5.00	80.00	<.001

Degree of freedom (df), probability (Pr). Variables include shoot dry weight (SDW), root dry weight (RDW), total root length (TRL), root-to-shoot ratio (RRS), and specific root length (SRL).

Table 2.6 Descriptive statistics for maize classification

Cluster	SDW (g)			RDW (g)			TRL (cm)			RRS			SRL (m g^{-1})		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
I	0.50	1.13	0.68b	0.34	0.56	0.44a	5591	7615	6554b	0.38	1.08	0.67a	125.7	184.1	154.1a
II	0.76	1.25	0.95a	0.41	0.61	0.50a	8125	9369	8657a	0.40	0.65	0.54a	153.8	214.1	182.7a
III	0.31	0.87	0.48c	0.15	0.35	0.23c	2038	3770	3140d	0.23	0.91	0.53a	99.5	194.6	153.4a
IV	0.32	1.18	0.61b	0.17	0.63	0.35b	4027	5429	4664c	0.24	1.19	0.65a	81.3	283.1	146.9a

Mean values in a column not followed by a common letter differ significantly ($p < 0.05$). Variables include shoot dry weight (SDW), root dry weight (RDW), total root length (TRL), root-to-shoot ratio (RRS), and specific root length (SRL).

2.4.3. Trait correlations

Pearson's correlation analysis for genotypes of soybean and maize showed that most traits had significant correlations under low P conditions. In soybeans, SDW had significant positive correlations with RDW, TRL, shoot and root P contents (R^2 = 0.90, 077, 0.91, and 0.66, respectively). RDW was strongly positively correlated with TRL, RRS, shoot and root P contents (R^2 = 0.86, 0.45, 0.83 and 0.85, respectively). TRL had significant positive correlations with SDW, RDW, RRS, shoot and root P contents (R^2 = 0.77, 0.86, 0.44, 0.74, and 0.75). Shoot P concentration was strongly negatively correlated with PUE (R^2 = -0.91) (Table 2.7).

In maize, SDW positively correlated with TRL and shoot P content (R^2 = 0.50 and 0.95, respectively) and negatively correlated with RRS and shoot P concentration (R^2 = -0.55 and -0.44). RDW had positive correlations with TRL, RRS, and shoot P content (R2 = 0.72, 0.55, and 0.32) and negatively correlated with SRL (R2 = -0.48). Shoot P concentration was strongly negatively correlated with PUE (R2 = -0.91) under low P conditions (Table 2.8).

2.5. Discussion

Cluster analysis of soybean and maize revealed that soybean cluster III and maize cluster II characterized the highest Cluster mean for SDW, RDW and TRL, indicating that the availability of promising genotypes for the performance under low P stress in these two cluster groups (Tables 2.4 and 2.6). This study found that traits such as shoot and root biomass, root length, and shoot and root P contents were highly correlated under low P availability (Table 2.7). The positive correlation between root dry weight and shoot and root P contents confirmed that genotypes with enhanced root growth under low P conditions could explore more nutrients (Tables 2.7 and 2.8). The cluster analysis was based on the traits SDW, RDW, TRL, RRS and SRL, widely used in assessing plant P efficiency (Zhao et al., 2021; Rose et al., 2016; Lu et al., 2009). However, further analysis is needed to test the selected genotypes under sufficient and deficient P conditions as a soil experiment.

Table 2.7 Pearson's correlation coefficients among selected parameters for soybean genotypes

	SDW	RDW	TRL	RRS	SRL	Shoot [P]	Root [P]	Shoot P content	Root P content	PUE
SDW	1.00									
RDW	0.90***	1.00								
TRL	0.77***	0.86***	1.00							
RRS	0.05ns	0.45***	0.44***	1.00						
SRL	-0.36*	-0.33*	0.04ns	-0.06ns	1.00					
Shoot [P]	-0.31*	-0.23*	-0.12ns	0.10ns	0.39***	1.00				
Root [P]	-0.39***	-0.23*	-0.17ns	0.23*	0.27*	0.58***	1.00			
Shoot P content	0.91***	0.83***	0.74***	0.08ns	-0.22*	0.09ns	-0.20ns	1.00		
Root P content	0.66***	0.85***	0.75***	0.57***	-0.16ns	0.09ns	0.24*	0.70***	1.00	
PUE	0.31*	0.24*	0.13ns	-0.11ns	-0.36***	-0.91***	-0.51***	-0.09ns	-0.04ns	1.00

Shoot dry weight (SDW), root dry weight (RDW), total root length (TRL), root-to-shoot ratio (RRS), specific root length (SRL), P concentration ([P]), phosphorus use efficiency (PUE). Correlation is significant if $p < 0.05$ (*) or $p < 0.001$ (***); not significant (ns).

Table 2.8 Pearson's correlation coefficients among selected parameters for maize genotypes

	SDW	RDW	TRL	RRS	SRL	Shoot [P]	Shoot P content	PUE
SDW	1.00							
RDW	0.30*	1.00						
TRL	0.50***	0.72***	1.00					
RRS	-0.55***	0.55***	0.12ns	1.00				
SRL	0.15ns	-0.48***	0.17ns	-0.61***	1.00			
Shoot [P]	-0.44***	-0.13ns	-0.06ns	0.20ns	0.11ns	1.00		
Shoot P content	0.95***	0.32*	0.55***	-0.53***	0.18ns	-0.18ns	1.00	
PUE	0.34*	0.05ns	0.00ns	-0.14ns	-0.07ns	-0.91***	0.05ns	1.00

Shoot dry weight (SDW), root dry weight (RDW), total root length (TRL), root-to-shoot ratio (RRS), specific root length (SRL), P concentration ([P]), phosphorus use efficiency (PUE). Correlation is significant if $p < 0.05$ (*) or $p < 0.001$ (***); not significant (ns).

Different screening methods are currently used for assessing P-efficient genotypes in different studies. We conducted the initial screening based on the selected traits only under low P conditions (Wang et al., 2021; Rose et al., 2010) based on the availability of seeds. However, the reality may be that P-efficient genotypes determined under P deficit conditions may not necessarily be responsive to adequate P conditions (Liang et al., 2023). Therefore, it is crucial to consider plant performances under both low and adequate P conditions.

We conducted the initial screening under hydroponic conditions. Although screening and selection under the appropriate field condition might be ideal, hydroponic culture systems are widely used (Cheng et al., 2014; Wang et al., 2013) and helpful in selecting a character impractical in the field, e.g., root morphology which plays an essential role in P uptake. However, hydroponic solutions were replaced at regular intervals or circulated from a large reservoir. Therefore, the low P stress experienced by plants growing under hydroponic conditions was not that low. In this study, the shoot P concentrations of soybean varied from 4 to 8 mg g^{-1} DW (Data not shown). P concentrations in agricultural crops generally vary from 1 to 5 mg g^{-1} DW (Anonymous, 1999). It further implied that the P concentration of the nutrient solution (50 µM) was sufficient for soybean growth. Compared to soybean, maize shoot P concentrations varied from 0.63 to 1.15 mg g^{-1} (Data not shown), indicating that maize plants grown under low P stress. Therefore, it is necessary to screen the selected cultivars under soil conditions with both sufficient and deficient conditions in the following experiments.

Investigating genotypic differences in response to low P stress, identifying P-efficient genotypes, and incorporating them in breeding programs are vital for sustainable agricultural development (Gu et al., 2016b; Liu et al., 2018b). Crop genotypes can be classified into two broad categories: "P-responsive" or "P-efficient." P-responsive genotypes produce higher yields under high P conditions, and P-efficient genotypes possess yield stability under low P conditions (Yaseen and Malhi, 2009). However, current commercial genotypes are P-responsive because they have been developed and selected on soils supplemented with enough P fertilizers with a focus on traits deemed more important than efficient P uptake and as a consequence, there has been a slight improvement in the P efficiency of crop genotypes over the past decades (Wissuwa et al., 2009). Further,

current commercial crop genotypes are relatively P-inefficient at accessing soil-bound P. They may only take up as little as 10 % of applied P fertilizer in the first year, with subsequent uptake rarely exceeding 50 % (Holford, 1997). In contrast, naturally existing low P-tolerant genotypes, including landraces and naturally inbred traditional cultivars, can withstand low P tolerance because they have been cultivated in P-deficient soils for an extended period. Genotypic variation exists in most crops, including maize (Aci et al., 2018; Li et al., 2021; Wang et al., 2019a) and soybean (Wang et al., 2010b, Ning et al., 2016, Zhou et al., 2016), suggesting that phosphorus efficiency can be improved by exploiting genotypic variation through breeding (Wissuwa et al., 2009).

2.6. Conclusions

According to the cluster analysis results, Japanese core collections of maize and soybean have considerable genotypic variation regarding low P tolerance. These genotypes may possess the genetic and phenotypic competence to withstand low P stress. Further, adaptations exhibited under low P stress may include PAE or PUE. Therefore, further research is needed to confirm their adaptations under low P stress while comparing growth performances under both deficient and sufficient P supply in selected cultivars of Japanese core collections of soybean and maize.

CHAPTER 3

SHOOT AND ROOT RESPONSES TO LOW P AND THEIR GENOTYPIC VARIABILITY IN SELECTED CULTIVARS OF JAPANESE CORE COLLECTIONS OF MAIZE AND SOYBEAN

3.1. Introduction

Phosphorus is the second most growth-limiting macronutrient in soils besides nitrogen due to its strong immobilization in soils (Kalayu, 2019). It involves several essential plant functions, such as energy transfers being part of ATP and NADP, as a structural component of nucleic acids; DNA, RNA, and a constituent of phospholipids, which are essential in cell membrane development and function (Khan et al., 2010; Lambers and Plaxton, 2018). Low P availability limits plant growth on many soils worldwide and negatively impacts agricultural productivity. Additionally, rock phosphate as raw material for P fertilizers is a non-renewable resource (Cordell et al., 2009). The remaining rock phosphate reserves are controlled by a handful of countries, including China, the United States of America, and Morocco (Cordell et al., 2009). The fertilizer industry recognizes that future phosphate reserves will likely yield low-quality phosphorus at a higher price due to the cost of extracting, processing, and shipping (Smil, 2000). Therefore, efficient utilization of P in soils is vital for sustainable agriculture.

To cope with P-deficient conditions, plants employ different mechanisms. Phosphorus-efficient genotypes are advanced in either PAE or PUE. PAE enables plants to explore large volumes of soil and thereby acquire more P from soil. PUE determined how acquired P is utilized efficiently for different plant functions and could bring a higher yield per unit of P acquired (Richardson et al., 2011). Strategies related to PAE and PUE are equally essential to improve the P efficiency of crops. However, their relative importance significantly depends on soil P status (Cong et al., 2020; Nadeem et al., 2022; Wang et al., 2010a). High P acquisition strategies have a crucial role in soils with high plant P availability through root foraging strategies and in soils where P is limited through root mining strategies. Most of these morphological, physiological, and biochemical strategies related to PAE are associated with crop genotypic variation (Dissanayaka et al., 2018; Liu et al., 2018b; Tesfaye, 2017; Wang et al., 2010a). Therefore, assessing crop species and their genotypic variability, understanding their genetic potential, and incorporating them in breeding programs are crucial for improving the low P-tolerant crop genotypes.

Root foraging strategies enable plants to develop a more exploratory root system under low P availability. Root architectural traits and the plastic nature of root growth

under low P conditions improve the PAE in crop genotypes (Dissanayaka et al., 2018; Furuya et al., 2022). P deprivation generally leads to a higher root-to-shoot ratio, changes in root extension (more lateral root growth and shallower root growth angles of axial roots), and modification in root hair growth (Niu et al., 2013). On the other hand, root mining strategies enhance the desorption and mineralization of sparingly available P and organic P pools through root exudates, organic anions, and phosphatases. Low P tolerance in maize (Aci et al., 2018; Li et al., 2021; Wang et al., 2019a) and soybean (Wang et al., 2010b, Ning et al., 2016, Zhou et al., 2016) have been reported in previous studies. Therefore, in this study, we aimed to evaluate the different shoot and root responses and their genotypic variability in selected landraces of two important food crops with divergent root traits under low P stress.

Maize, a typical monocot, has a fibrous root system, whereas soybean, a typical dicot, is a tap-root crop. A sufficient level of P in soil solution is pivotal for both species' optimum growth and yield. Further, these crop species are essential in diversified cropping systems like intercropping or rotations. Cereal/legume intercropping is a classic combination at present. Accordingly, genotype strategies could be combined with agronomic strategies to enhance P efficiency jointly (Cong et al., 2020). In diversified cropping systems like intercropping, P efficiency can be effectively enhanced by including genotypes characterized by high P acquisition or P mobilizing. Including such efficient genotypes expected to be facilitators for the intercropped companion species under low P soil conditions through facilitative interactions.

Plants have evolved adaptive mechanisms to overcome low P availability to maintain P homeostasis. One of the main mechanisms is to maximize the ability of the root to uptake P from the soil. The modification of root architecture is a powerful tool for developing crop plants with an efficient P acquisition ability. Root architecture is a highly plastic trait and varies among species and cultivars in response to low P conditions. The root architecture of monocots and dicots differs significantly, where monocot roots are fibrous, and dicot roots are taproots. However, all vascular plant species share the main adaptive root traits for enhancing P acquisition (Niu et al., 2013). Despite significant progress in understanding plant strategies associated with P mobilization and acquisition, each plant species and cultivar responds differently to P supply. This study investigated

differences in response to P deficiency in maize and soybean, particularly regarding root morphology and rhizosphere soil activity. We compared plant growth, biomass allocation, rhizosphere soil activities and P use efficiency between maize and soybean using ten cultivars of each species, including low P-tolerant, moderately tolerant, and sensitive cultivars grown under P deficient conditions in Regosols. The following questions were addressed through the comparison: (1) Does biomass allocation and P accumulation change in response to low P availability in these two species? (2) Does the P use efficiency of maize differ from that of the soybean under low P stress? (3) Are the rhizosphere soil activities of maize different from those of soybean under P deficiency?

3.2. Objectives

We aimed to evaluate selected cultivars in maize and soybean Japanese core collections for different responses and adaptations under P deficient and sufficient conditions, using a pot experiment at the early growth stage. This study characterized the shoot and root responses to low P to identify P-efficient genotypes among the tested cultivars and their genotypic variation in low P tolerance. We hypothesized that (1) low P stress reduces shoot growth but stimulates root growth in both species, (2) the plasticity of the root system and PUE are critical to withstand low P conditions, and (3) there would be a considerable genotypic variation among the tested genotypes of both species regards to their shoot and root responses under low P stress.

3.3. Materials and methods

The study was comprised of two separate pot experiments for two species, soybean and maize, under soil conditions. Ten cultivars of each maize and soybean in the Japanese core collections were selected based on the preliminary screening results under hydroponic conditions and further evaluated under soil conditions. A complete list of landraces examined in this study is shown in Table 3.1. Experimental IDs were based on the preliminary screening test (Table 3.1).

3.3.1. Selection of cultivars from preliminary screening

Based on the results, maize and soybean core collections were clustered into 4 groups (Figures 2.1, 2.2; Tables 2.4 and 2.6). From each cluster, 2-3 representative cultivars (in

total, 20 cultivars) were selected for further assessment (Tables 3.2 and 3.3). According to the cluster descriptives of maize, the selection of cultivars from Cluster II and III was based on the maximum and minimum values of SDW, respectively (Tables 2.6 and 3.2). The cultivar selection pattern for the rest of the clusters (I and IV) was based on maximum values of SDW (Tables 2.6 and 3.2).

According to the cluster descriptives of soybean, the SDW values ranged from 0.45 to 2.51 g/plant in Cluster IV (Table 2.4), and three cultivars selected from Cluster IV represented that range's lower, middle and upper levels (Table 3.3). The cultivar selection pattern for the rest of the clusters was based on maximum values of SDW (Tables 2.4 and 3.3).

3.3.2. Pot experiment

The pot experiment was conducted in the glasshouse at Hiroshima University, Japan. The Regosols used in this study were collected from the Fukuyama area in Hiroshima prefecture, Japan. The soil was air-dried and sieved to 2 mm. This study used two P supply rates: low P (10 mg P kg^{-1} dry soil) and high P (50 mg P kg^{-1} dry soil). $NaH_2PO_4.2H_2O$ was used as the P source, and soil was supplied with relevant P rates with 100 mg N kg^{-1} dry soil as NH_4NO_3, 100 mg K kg^{-1} dry soil as K_2SO_4 and 1000 mg Ca/Mg kg^{-1} dry soil as $CaMg(CO_3)_2$. The plastic pots were filled with 2 kg of prepared soil. After filling, pots were adjusted for soil moisture between 40-60 % of water holding capacity (WHC) (0.38 L /kg). Each cultivar consisted of four replicates, and each species had 80 pots. In total, 160 pots were maintained for the whole study. Two seeds of each cultivar were sown in each pot, and after one week of germination, one seedling was thinned out. In this study, plants were harvested 30 days after seed sowing. The average glasshouse temperature for the duration of the experiment was 30/25 ºC (day/night) with a photoperiod of 14h day/10h night.

3.3.3. Plant and soil analysis

At harvest, the shoot and roots were separated. The root systems were lifted carefully out of the soil with minimal damage. The root systems were shaken gently to remove loosely adhering soil. Then, the rhizosphere soil samples were collected by shaking the root systems vigorously. Immediately after collection, rhizosphere soil samples were sieved to 2

mm to remove root debris and stored at 4 ºC for the acid phosphatase (ACP) analysis. The root systems were thoroughly washed with tap water on a 0.5 mm mesh screen and stored in distilled water-filled plastic containers at 4 ºC until root scanning. Shoots were oven-dried at 80 ºC for three days. The roots were cut into segments and floated in a transparent acrylic tray for digital images using an image scanner (Epson Gt-X970, Seiko Epson Corp., Nagano, Japan). Using the free software ImageJ (https://imagej.nih.gov/ij/), the TRLs of the scanned images were calculated. After root scanning, root samples were oven-dried at 80 ºC for three days. Shoot dry weight (SDW), root dry weight (RDW), RRS, and SRL were evaluated. SRL is the ratio of TRL to RDW. All dried shoot and root samples were ground. Plant samples (200 mg) were digested for P determination using the HNO_3 and H_2O_2 digestion method described in Wheal et al. (2011). The P concentrations of extracts were quantified spectrophotometrically (UV-1800, Shimadzu Corporation, Kyoto, Japan) using the phosphomolybdate-blue method (Murphy and Riley, 1962). P contents in the shoot (or root) were calculated by multiplying the shoot (or root) dry weight with P concentration in the shoot (or root). PUE (dry weight per unit P uptake) was calculated as shoot DW divided by shoot P content (Moll et al., 1982). The ACP activity of rhizosphere soil was determined based on the published method described by Olinger et al. (1996). This method was a modified method of the original methods by Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1977). According to this method, 1 g of moist soil was incubated in a modified universal buffer (pH 6.5) with p-nitrophenyl phosphate at 37 ºC for 1 hour (h), and p-nitrophenol (NP) released by phosphomonoesterase activity was measured using a spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan) at 400 nm. ACP activity of rhizosphere soil measured using moist soil was converted to ACP activity per dry weight of soil considering the moisture content of soil and finally expressed as ($\mu g\ NP\ g^{-1}\ DW\ h^{-1}$). Rhizosphere soil pH was measured in a 1:2.5 soil-to-water suspension.

3.3.4. Soil properties

The physical and chemical properties of the Regosols used in this study are presented in Table 3.4. Soil pH was determined in a 1:2.5 soil-to-water suspension using a pH meter. Soil total N and C were determined by a CN analyzer (MT-700; Yanaco Kyoto, Japan). The available soil P was determined colorimetrically using the Bray II method (Bray and

Kurtz, 1945). Soil samples (0.2 g) were digested with a 1:1 mixture of concentrated H_2SO_4 and HNO_3 (10 mL) in a Kjeldahl flask for total P analysis. P content was determined spectrophotometrically (UV-1800, Shimadzu Corporation, Kyoto, Japan) using the phosphomolybdate-blue method (Murphy and Riley, 1962). The P absorption coefficient was analyzed according to methods described by Sekiya (1970).

3.3.5. Statistical analysis

Data were subjected to two-way analysis of variance (ANOVA) in SPSS software (IBM SPSS Statistics for Windows, Version 28.0.0.0. Armonk, NY: IBM Corp) to examine the impact of cultivars and P supply (low P and high P) and their interactions on response variables separately for maize and soybean. Tukey's Studentized Range Test calculated significant differences between means at the 0.05 probability level. Separate correlation matrix-based principal component analyses (PCA) were conducted for maize and soybean on variables to characterize the cultivars under low and high P supply. Before PCA, all variable data were standardized by calculating Z-score values. Mathematically, this was done by subtracting the mean and then dividing it by the standard deviation for each observed value of each variable (resulting in all variables having a mean of zero and a variance of 1). Graphs were plotted using R software (R Core Team, 2022) and ggplot packages.

For the species comparison, SDW, RDW, TRL, RRS, SRL, shoot and root P concentrations, shoot and root P contents, rhizosphere soil pH and ACP, and PUE under low P and high P conditions were taken into consideration by taking the mean value of ten cultivars for each parameter. Data were subjected to two-way ANOVA in SPSS software (IBM SPSS Statistics for Windows, Version 28.0.0.0. Armonk, NY: IBM Corp) to examine the impact of species and P supply (low P and high P) and their interactions on response variables. Tukey's Studentized Range Test calculated significant differences between means at the 0.05 probability level.

Table 3.1 Summary of the cultivars used in the pot experiment

Experimental ID	Gene bank ID	Name	Origin
Maize			
76	JMC 76	IWANAI ZAIRAI B	Hokkaido (Japan)
42	JMC 42	TANOHATA 1	Iwate (Japan)
57	JMC 57	TORIYABE	Aomori (Japan)
58	JMC 58	SHIONOMATA 1	Fukushima (Japan)
78	JMC 78	IWANAI ZAIRAI	Hokkaido (Japan)
8	JMC 8	SHIMONAKA MURAKAMI	Ehime (Japan)
13	JMC 13	IIBOSHI 2	Miyazaki (Japan)
71	JMC 71	YUUBARI ZAIRAI A	Hokkaido (Japan)
75	JMC 75	KAMEDA ZAIRAI C	Hokkaido (Japan)
80	JMC 80	SAKASHITA TAIKI	Hokkaido (Japan)
Soybean			
42	GmJMC064	MEGURO 1	Aomori (Japan)
37	GmJMC059	SOKOSHIN	Niigata (Japan)
18	GmJMC033	BANSEI HIKARIKURO	Hokkaido (Japan)
56	GmJMC090	DADACHAMAME	Yamagata (Japan)
67	GmJMC102	KURAKAKE	Niigata (Japan)
74	GmJMC114	COL/EHIME/1-2	Ehime (Japan)
83	GmJMC137	COL/EHIME/1983/UTSUNOMIYA 37	Ehime (Japan)
22	GmJMC040	KOIBUCHIMURA ZAIRAI	Ibaraki (Japan)
54	GmJMC085	DAIZU	Wakayama (Japan)
70	GmJMC106	HIMESHIRAZU	Chiba (Japan)

Table 3.2 Shoot and root growth responses of selected maize cultivars from preliminary screening

Cluster	Cultivar	SDW (g)	RDW (g)	TRL (cm)	RRS	SRL (m g^{-1})
I	76	1.13 ± 0.07	0.44 ± 0.05	5966 ± 1204	0.39 ± 0.03	128.7 ± 25.8
	42	0.60 ± 0.06	0.39 ± 0.03	7151 ± 412	0.65 ± 0.03	181.7 ± 9.8
II	57	1.25 ± 0.12	0.56 ± 0.04	8662 ± 1467	0.46 ± 0.05	171.4 ± 34.9
	58	0.97 ± 0.08	0.61 ± 0.07	8706 ± 1176	0.63 ± 0.04	153.8 ± 13.5
	78	1.03 ± 0.05	0.41 ± 0.04	8613 ± 802	0.40 ± 0.07	214.1 ± 18.3
III	8	0.44 ± 0.06	0.35 ± 0.07	3140 ± 343	0.77 ± 0.07	113.3 ± 9.8
	13	0.38 ± 0.04	0.30 ± 0.05	2825 ± 61	0.79 ± 0.05	99.5 ± 18.0
	71	0.45 ± 0.05	0.16 ± 0.03	3254 ± 361	0.37 ± 0.03	194.6 ± 16.1
IV	75	1.18 ± 0.14	0.32 ± 0.02	4885 ± 439	0.27 ± 0.02	144.1 ± 17.5
	80	0.82 ± 0.10	0.23 ± 0.02	4240 ± 403	0.27 ± 0.02	190.9 ± 16.9

Values represent the mean of four replicates ± standard error (S.E.). Traits include shoot dry weight (SDW), root dry weight (RDW), total root length (TRL), root-to-shoot ratio (RRS), and specific root length (SRL).

Table 3.3 Shoot and root growth responses of selected soybean cultivars from preliminary screening

Cluster	Cultivar	SDW (g)	RDW (g)	TRL (cm)	RRS	SRL (m g^{-1})
I	42	2.27 ± 0.18	0.39 ± 0.02	4113 ± 262	0.17 ± 0.01	105.4 ± 3.0
	37	2.42 ± 0.12	0.51 ± 0.06	4872 ± 183	0.21 ± 0.02	107.6 ± 2.5
II	18	2.62 ± 0.14	0.47 ± 0.03	5055 ± 23	0.18 ± 0.02	109.0 ± 5.4
	56	2.38 ± 0.30	0.56 ± 0.08	6341 ± 1194	0.24 ± 0.01	111.2 ± 6.0
III	67	3.18 ± 0.39	0.93 ± 0.20	9040± 746	0.29 ± 0.04	125.8 ± 17.9
	74	2.96 ± 0.30	0.66 ± 0.04	8731 ± 977	0.23 ± 0.01	131.0 ± 8.0
	83	2.86 ± 0.50	0.64 ± 0.06	8356 ± 1255	0.23 ± 0.02	119.8 ± 9.8
IV	22	2.51 ± 0.33	0.39 ± 0.09	3269 ± 479	0.15 ± 0.02	94.2 ± 5.9
	54	1.48 ± 0.08	0.26 ± 0.02	3373 ± 500	0.18 ± 0.01	128.1 ± 15.6
	70	0.90 ± 0.22	0.16 ± 0.04	1881 ± 586	0.17 ± 0.01	130.4 ± 4.8

Values represent the mean of four replicates ± standard error (S.E.). Traits include shoot dry weight (SDW), root dry weight (RDW), total root length (TRL), root-to-shoot ratio (RRS), and specific root length (SRL).

Table 3.4 Physical and chemical properties of the soil used in the study

Soil type	Texture	Coarse sand	Fine sand	Silt	Clay	pH (1:2.5, H_2O)	Total C	Total N	Available P (Bray II)	Total P	P absorption coefficient
		%					g kg^{-1}		mg kg^{-1}		g P_2O_5 kg^{-1}
Regosols	Sand	67.9	25.6	5.4	1.1	5.8	2.2	0.5	6.4	169	2.49

Values are expressed on an oven-dry basis.

3.4. Results

3.4.1. Shoot and root growth responses of maize

There was a significant interaction between genotype and P supply regarding SDW, RDW, TRL, RRS and SRL. It further indicates that any differences in the above variables among maize cultivars differ for P supply. Among the tested maize cultivars, except JMC 76, all other cultivars reduced the shoot biomass drastically in low P (Figure 3.1(a)). Under low P, the recorded shoot dry weights for the cultivars JMC 57, JMC 76, JMC 78 and JMC 80 were significantly higher than those recorded by cultivars JMC 13 and JMC 71.

Nevertheless, there was also a variation in shoot dry weights under the high P. The highest shoot dry weight was recorded for the cultivar JMC 8, which is not statistically different from JMC 78, JMC 57, JMC 80, and JMC 58. JMC 71 resulted in the least dry weight under high P. RDW was not significantly different between high P and low P except in cultivars JMC 8, JMC 13 and JMC 80 (Figure 3.2(a)). A distinct reduction in RDW can be observed with P deprivation in these three cultivars. The highest RDW was recorded for JMC 8 under both P supply rates, but it was only statistically different from three cultivars: JMC 71, JMC 75 and JMC 80 under low P. The lowest RDW was found in JMC 71 for both low and high P, not statistically different from the cultivars JMC 42, JMC 75 and JMC 80 under low P. To TRL, variability could be observed among the cultivars at each P supply. The cultivars JMC 58, JMC 76, JMC 8 and JMC 57, which recorded higher TRLs in low P treatment, could enhance or keep a similar extension in TRL even under low P, where only JMC 76 resulted in a significant enhancement compared to high P (Figure 3.3(a)). In JMC 80, the decline in TRL was significant compared to high P. Again, the lowest value for TRL was found in JMC 71 under both P supply rates, not statistically different from JMC 13 and JMC 75 under low P. Low P caused the rise in RRS, significant in all cultivars except for JMC 75 and JMC 80 (Figure 3.4(a)). SRL also significantly increased under the low P only in JMC 76, JMC 42, JMC 58, JMC 78 and JMC 8 (Figure 3.5(a)). Hence, genotypic variation was observed in the shoot and root responses under both P conditions (Figures 3.1-3.5 (a)).

High P acquisition strategies are crucial in soils with high available P and desirable for soils with lower available P. According to the above results, JMC 57, JMC 58 and JMC 8 seemed stronger in P acquisition under both P regimes due to their root traits. The

cultivar JMC 76 seemed only vigorous in an acquisition under low P. In contrast, JMC 71 showed weak growth under both P levels.

3.4.2. Shoot and root growth responses of soybean

There was a noticeable interaction between cultivar and P supply for RDW, RRS and SRL for soybean. Consequently, any differences among cultivars on the above variables differed at the P supply rate. Like maize results, genotypic variation could be observed in shoot and root growth responses among selected soybean cultivars under high P and lower P (Figures 3.1-3.5(b)). Shoot biomass was markedly reduced with P deprivation in all cultivars except GmJMC033 (Figure 3.1(b)). In GmJMC033, the biomass reduction was 19%, and the highest cutdown (51%) was recorded for GmJMC064 and GmJMC106. GmJMC106 resulted in the lowest SDW value for high P, which is not statistically different from GmJMC059. With the P impoverishment, all cultivars increased RDW except GmJMC064, GmJMC059 and GmJMC106, which decreased RDW, though the decrease was insignificant compared to high P (Figure 3.2(b)). TRL was not significantly different between the two P supply rates in all cultivars except in GmJMC040 and GmJMC085 (Figure 3.3(b)). These two cultivars markedly increased root extension under low soil P conditions, and GmJMC085 recorded the highest TRL under both soil P supplies. P deficit increased the RRS in all cultivars, but the increment was insignificant in GmJMC059 and GmJMC106 compared to high P (Figure 3.4(b)). In low P treatment, SRL was notably higher in GmJMC085 and GmJMC106 (Figure 3.5(b)). These two cultivars had contradictory responses under low P, whereas GmJMC085 recorded the highest RDW and TRL, and GmJMC106 recorded the lowest RDW and TRL under low P. At the same time, GmJMC085 resulted in the highest SRL, which is not statistically different from GmJMC090 under high P. Further, its SRL value under low P was not significantly different from high P.

Consequently, GmJMC085 seems stronger in P acquisition under P deficit due to its root traits. Contrary to GmJMC085, GmJMC106 showed poor growth under both P conditions. Therefore, a significant genotypic correlation between some root traits and biomass production under P-limiting conditions may be the reason for such variations.

Table 3.5 Significance of different sources of variance

Parameter	Source of variability					
	Maize			Soybean		
	C	P	C × P	C	P	C × P
Shoot dry weight (g plant^{-1})	<0.001	<0.001	<0.001	<0.001	<0.001	0.272
Root dry weight (g plant^{-1})	<0.001	0.021	0.048	<0.001	0.003	<0.001
Total root length (cm plant^{-1})	<0.001	0.439	0.011	<0.001	0.035	0.115
Root-to-shoot ratio	<0.001	<0.001	0.022	<0.001	<0.001	0.021
Specific root length (m g^{-1})	<0.001	<0.001	0.006	<0.001	0.751	0.003
Shoot P concentration (mg g^{-1})	<0.001	<0.001	0.002	<0.001	<0.001	0.017
Root P concentration (mg g^{-1})	<0.001	<0.001	0.057	0.364	<0.001	0.002
Shoot P content (mg P plant^{-1})	<0.001	<0.001	0.002	0.002	<0.001	0.075
Root P content (mg P plant^{-1})	<0.001	<0.001	0.437	<0.001	<0.001	<0.001
Phosphorus use efficiency (g DW g^{-1} P)	0.018	<0.001	0.458	<0.001	<0.001	0.075
Acid phosphatase activity (µg NP g^{-1} DW h^{-1})	<0.001	<0.001	<0.001	0.003	<0.001	0.089
Rhizosphere pH	0.125	<0.001	<0.058	0.481	<0.001	.009

Probability values in the table are related to a two-way analysis of variance for the factors cultivar (C), phosphorus supply (P), and the interaction of cultivar × phosphorus supply (C × P) at the significance level of ($p < 0.05$)

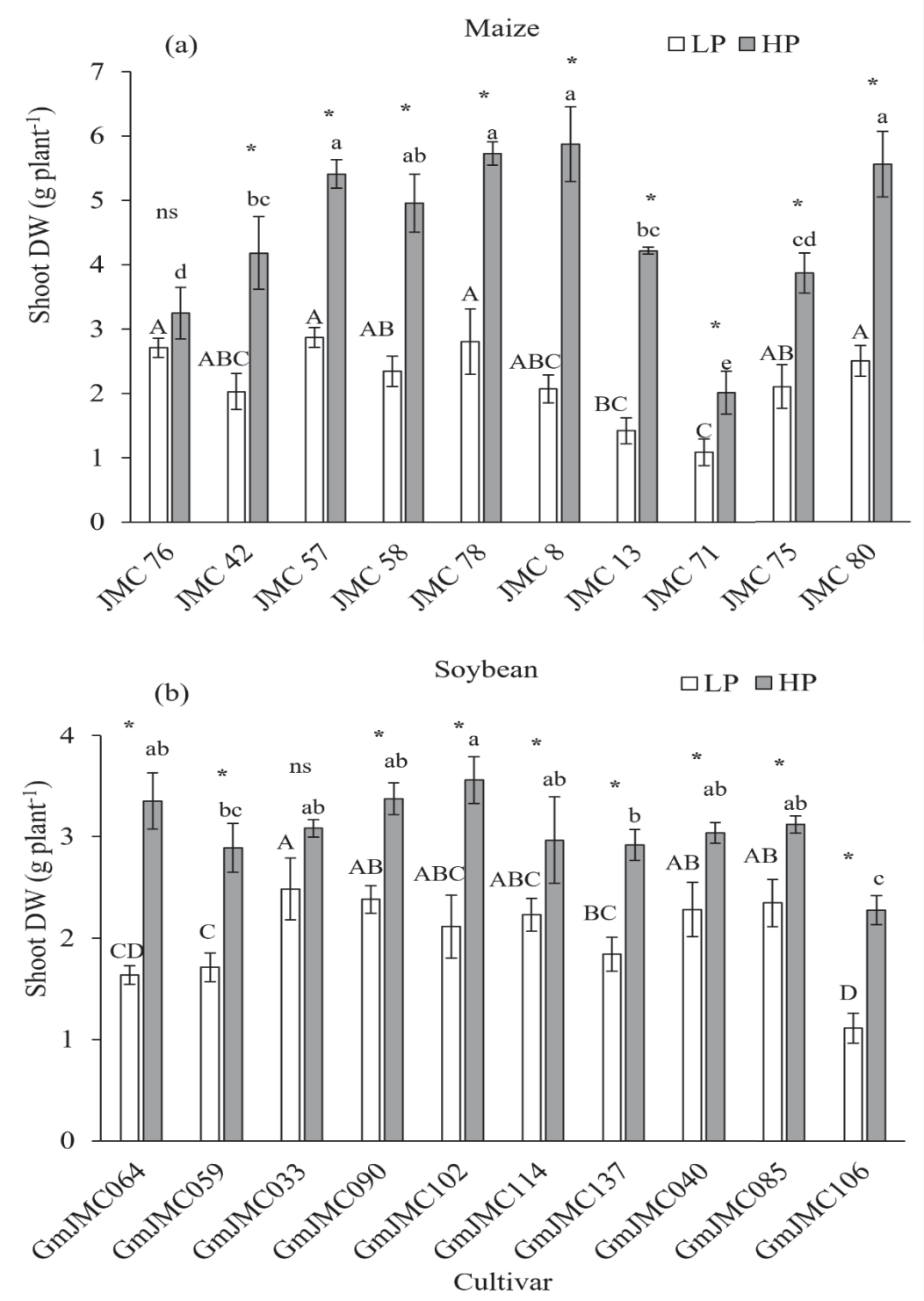

Figure 3.1 Shoot DW of maize (a) and soybean (b) cultivars under low P and high P conditions. Values not followed by a common letter in each cultivar at each P supply differ significantly ($p < 0.05$). Error bars represent the standard error (S.E.; n = 4). Cultivars indicated with an asterisk are significantly different ($p < 0.05$) in between low P and high P; ns (not significant).

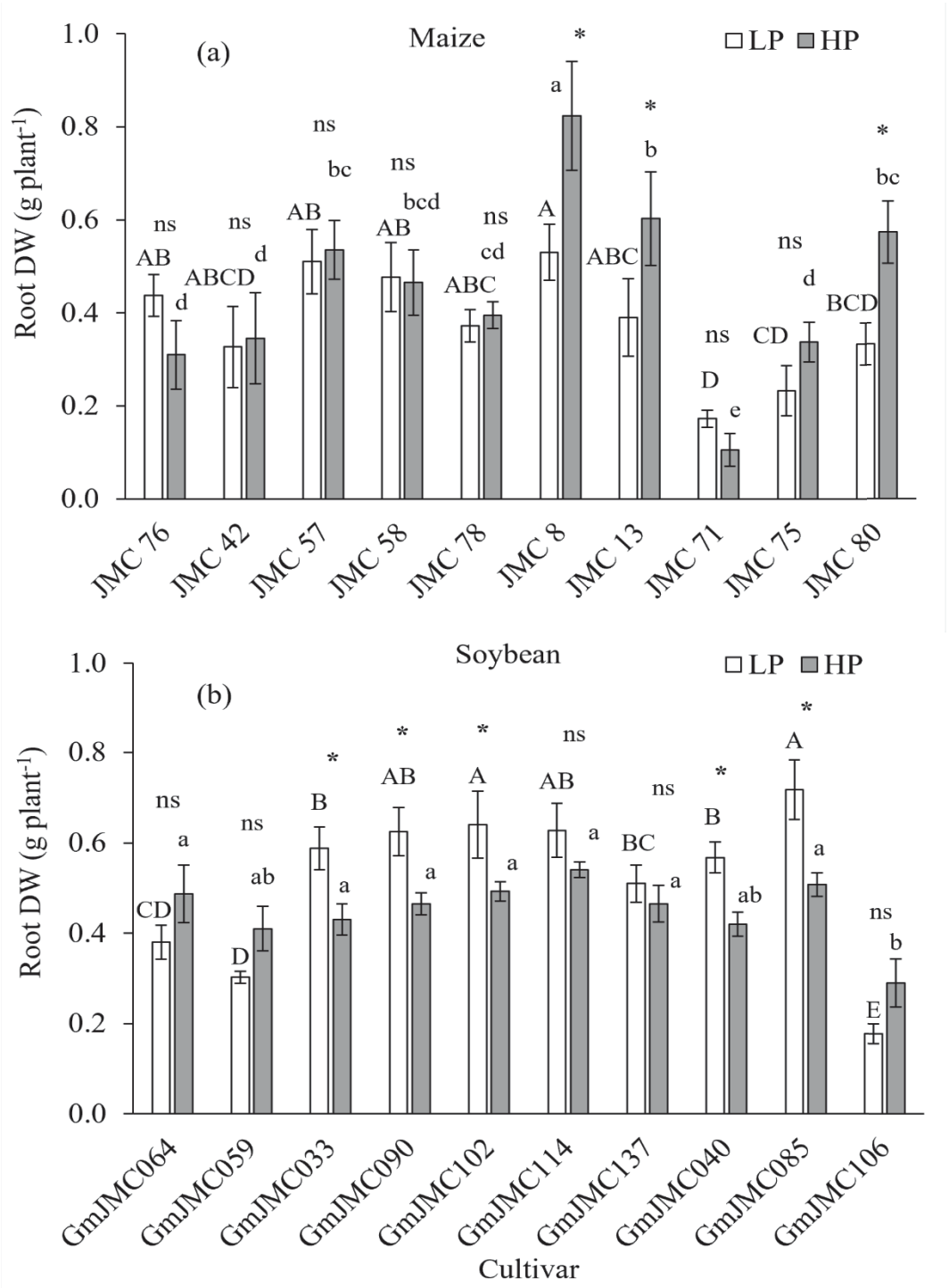

Figure 3.2 Root DW of maize (a) and soybean (b) cultivars under low P and high P conditions. Values not followed by a common letter in each cultivar at each P supply differ significantly ($p < 0.05$). Error bars represent the standard error (S.E.; n = 4). Cultivars indicated with an asterisk are significantly different ($p < 0.05$) in between low P and high P; ns (not significant).

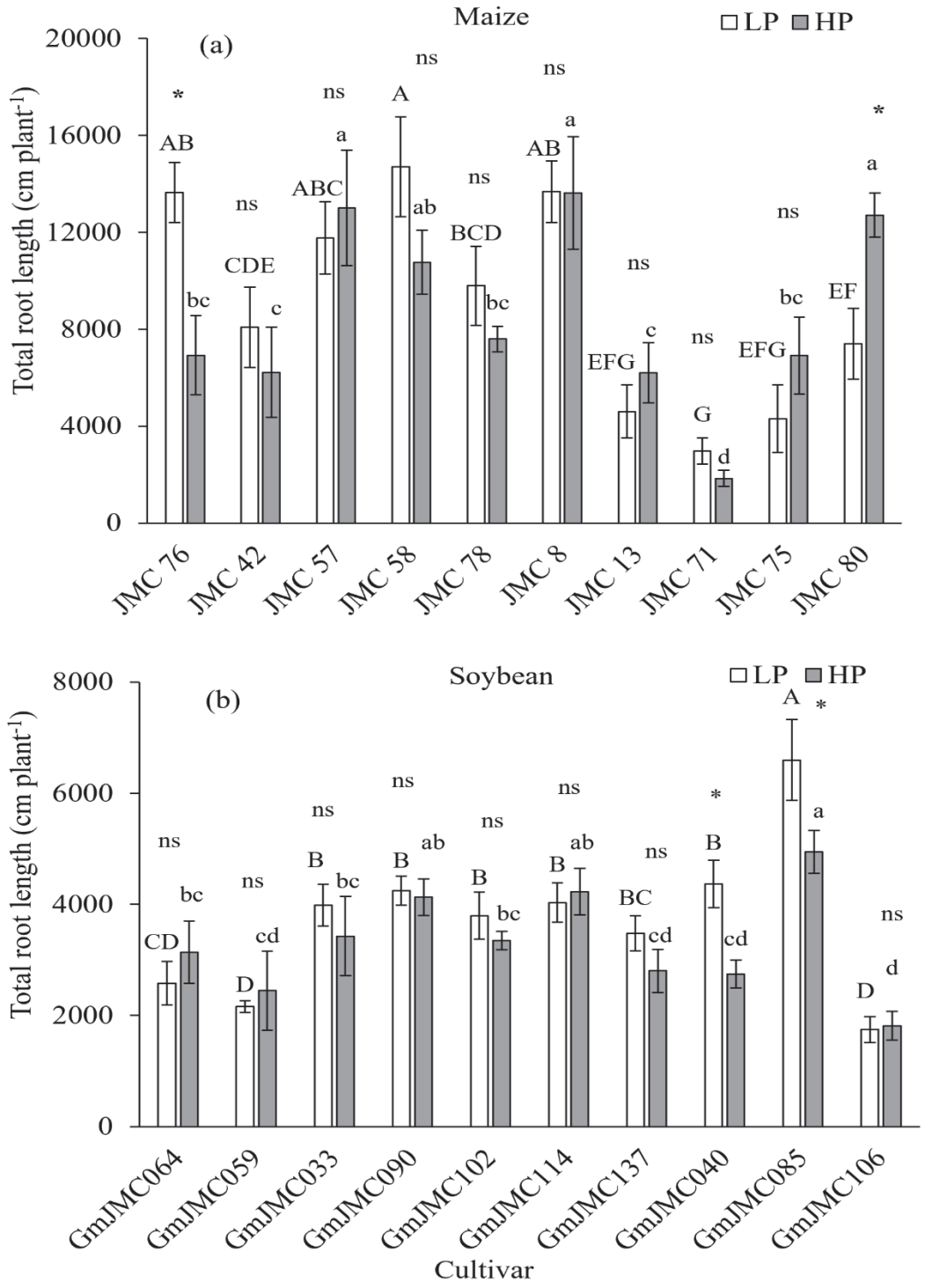

Figure 3.3 Total root length of maize (a) and soybean (b) cultivars under low P and high P conditions. Values not followed by a common letter in each cultivar at each P supply differ significantly ($p < 0.05$). Error bars represent the standard error (S.E.; n = 4). Cultivars indicated with an asterisk are significantly different ($p < 0.05$) in between low P and high P; ns (not significant).

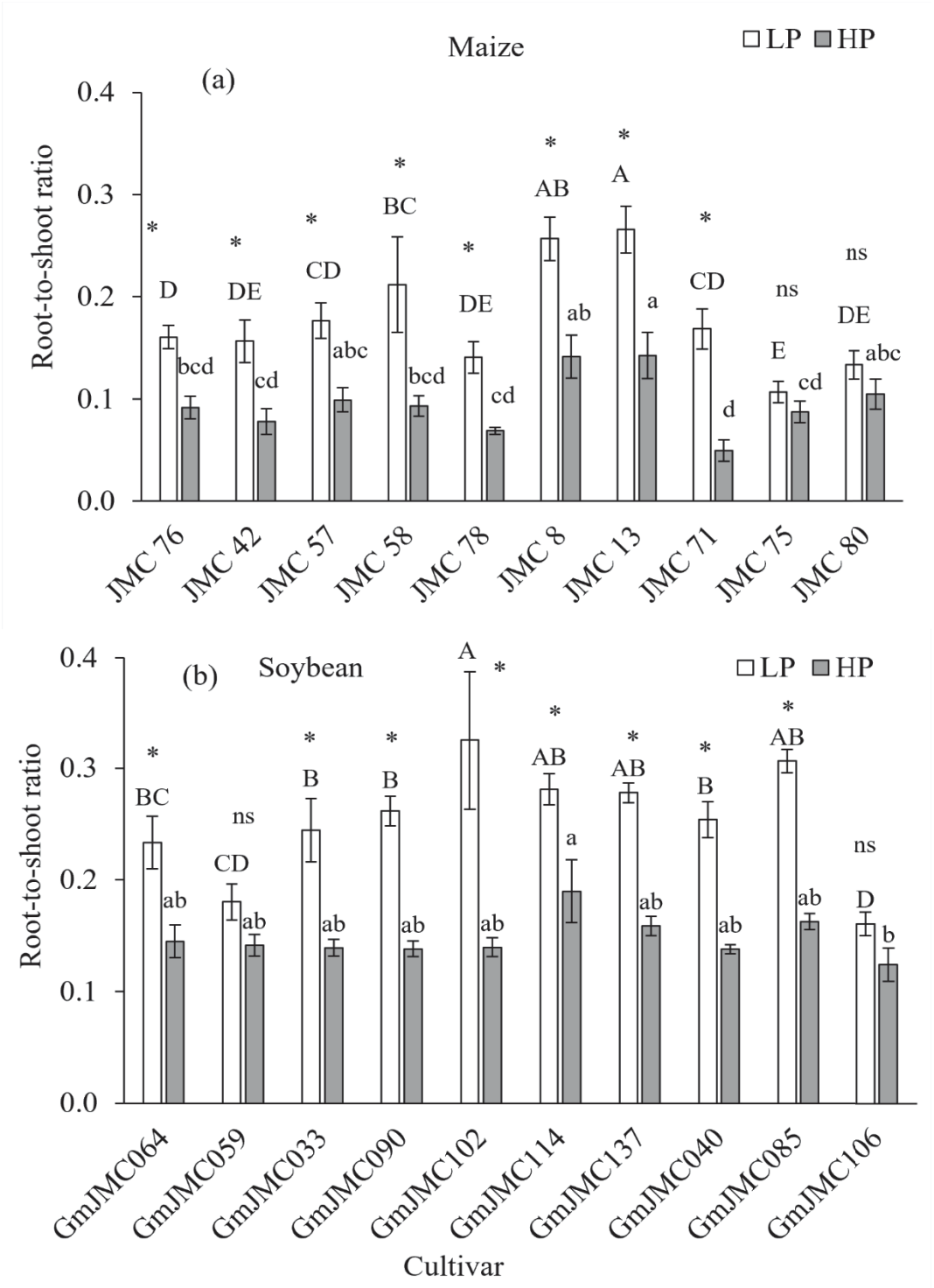

Figure 3.4 Root-to-shoot ratio of maize (a) and soybean (b) cultivars under low P and high P conditions. Values not followed by a common letter in each cultivar at each P supply differ significantly ($p < 0.05$). Error bars represent the standard error (S.E.; n = 4). Cultivars indicated with an asterisk are significantly different ($p < 0.05$) in between low P and high P; ns (not significant).

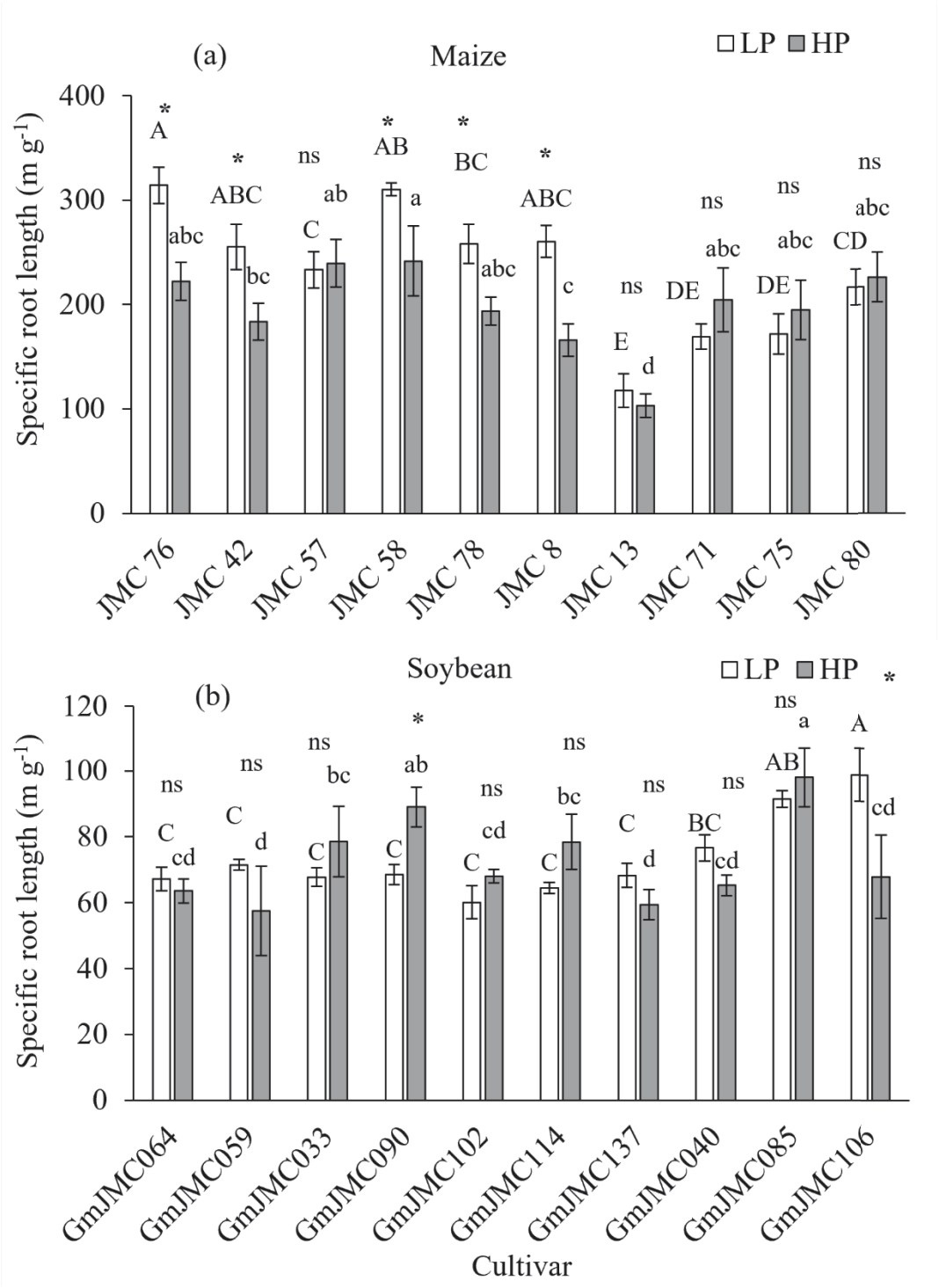

Figure 3.5 Specific root length of maize (a) and soybean (b) cultivars under low P and high P conditions. Values not followed by a common letter in each cultivar at each P supply differ significantly ($p < 0.05$). Error bars represent the standard error (S.E.; n = 4). Cultivars indicated with an asterisk are significantly different ($p < 0.05$) in between low P and high P; ns (not significant).

3.4.3. P accumulation and PUE

Both shoot and root P concentrations and P contents in shoots were significantly lower in low P treatment in maize cultivars (Figures 3.6(a and b) and 3.7(a)). In shoot P concentrations and contents, the genotypic variation could be found under the high P but not under the low P (Figures 3.6(a) and 3.7(a)). Under the high P, shoot P content was more remarkable in maize cultivars: JMC 57, JMC 78, JMC 58, JMC 80 and JMC 8 (Figure 3.7(a)). The genotypic variations in root P concentrations were observed in both high P and low P (Figure 3.6(b)). Higher root P concentrations were recorded in low P treatment for maize cultivars JMC 42, JMC 75, and JMC 13, which were not statistically different from JMC 71 and JMC 8. The root P content was highest in JMC 8 in both low P and high P, though it was insignificant with JMC 13 and JMC 57 under low P (Figure 3.7(b)). Root P content was significantly decreased under low P in cultivars except JMC 13, JMC 71, JMC 75 and JMC 80.

The shoot P concentrations in soybean cultivars were significantly lower in low P treatment than in high P in all cultivars except GmJMC059 and GmJMC106 (Figure 3.6(c)). The genotypic variation in shoot P concentrations was notable in high P. The highest shoot P concentrations were recorded in GmJMC085 for high P, which is not statistically different from GmJMC137, and in GmJMC106 for low P treatment. However, no statistically significant difference was found in shoot P concentrations among GmJMC085, GmJMC106, GmJMC059 and GmJMC137 in low P treatment. The shoot P content was not significantly different among soybean cultivars under low P, but notable variation could be found under high P (Figure 3.7(c)). P scarcity caused a significant reduction in shoot P accumulation in soybeans. The highest shoot P content resulted in under high P, which is insignificant from GmJMC137.

Although root P concentrations were significantly lower in low P compared to high P, there were no significant differences among the cultivars (Figure 3.6(d)). In high P treatment, there was a considerable genotypic difference in root P concentrations, and the highest root concentration was recorded in cultivar GmJMC137, which was not statistically different from GmJMC085 and GmJMC064. Root P content decreased significantly under the low P in all cultivars except GmJMC040 (Figure 3.7(d)). Genotypic variation was noticeable in root P content under both high and low P.

P deprivation caused a significant increase in the PUE in both maize and soybean (Figure 3.8). Genotypic variation could be found under low P in maize and soybeans. In maize, PUE was highest in JMC 80, not statistically different from JMC 57, JMC 78, JMC 13 and JMC 71 under low P (Figure 3.8(a)). In soybean cultivars GmJMC059 and GmJMC106, PUEs were not significantly different between high P and low P (Figure 3.8(b)). GmJMC085 also resulted in comparatively lower PUE under low P treatment. PUE was significantly higher in GmJMC064 in low P conditions than in other soybean cultivars.

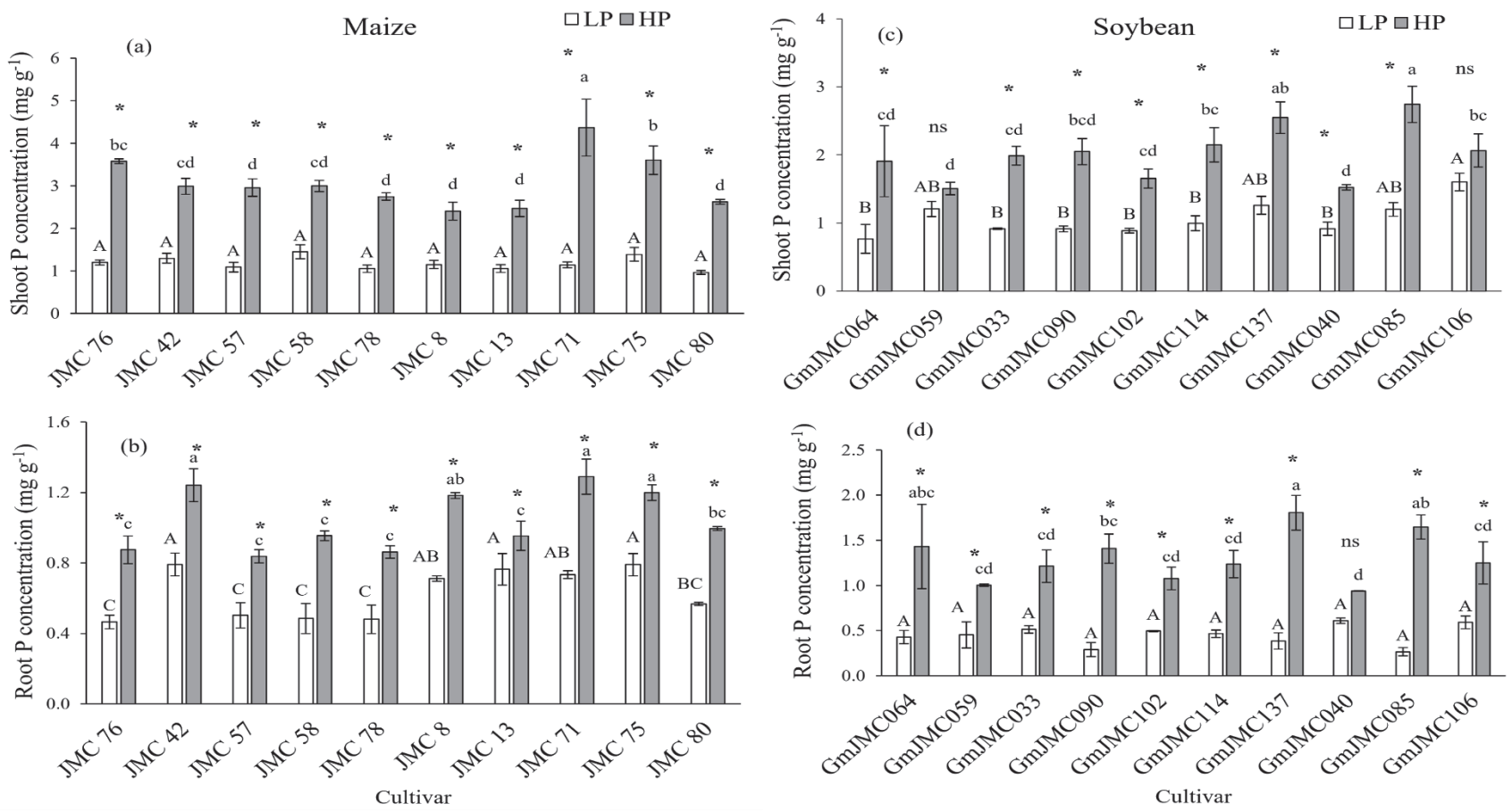

Figure 3.6 P concentrations of shoot and roots in maize and soybean cultivars under low P and high P conditions. (a) shoot P concentration of maize, (b) root P concentration of maize, (c) shoot P concentration of soybean, (d) root P concentration of soybean. Values not followed by a common letter in each cultivar at each P supply differ significantly ($p < 0.05$). Error bars represent the standard error (S.E.; n = 4). Cultivars indicated with an asterisk are significantly different ($p < 0.05$) in between low P and high P; ns (not significant).

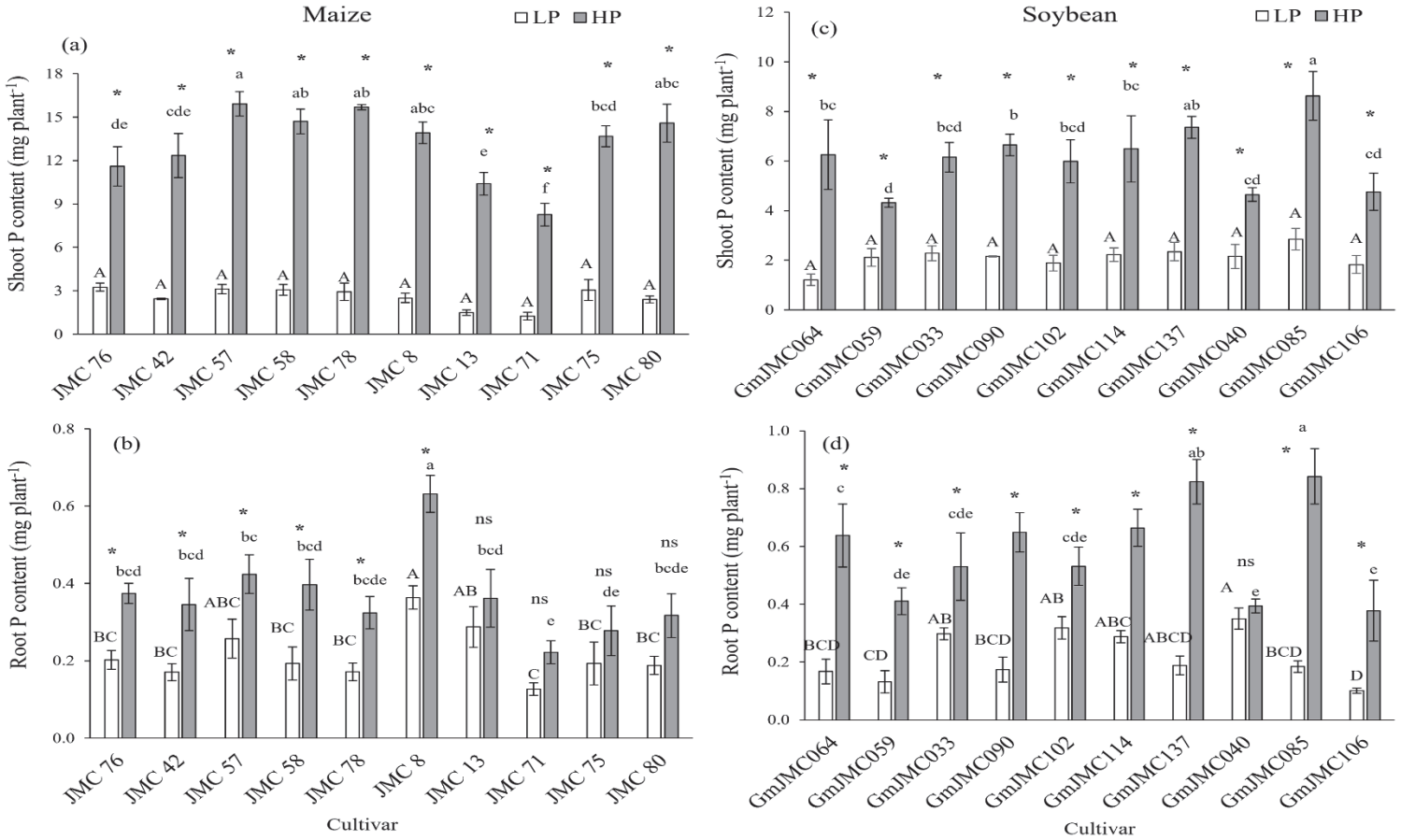

Figure 3.7 P accumulation in shoot and roots in maize and soybean cultivars under low P and high P conditions. (a) shoot P content of maize, (b) Root P content of maize, (c) shoot P content of soybean, (d) Root P content of soybean. Values not followed by a common letter in each cultivar at each P supply differ significantly ($p < 0.05$). Error bars represent the standard error (S.E.; n = 4). Cultivars indicated with an asterisk are significantly different ($p < 0.05$) in between low P and high P; ns (not significant).

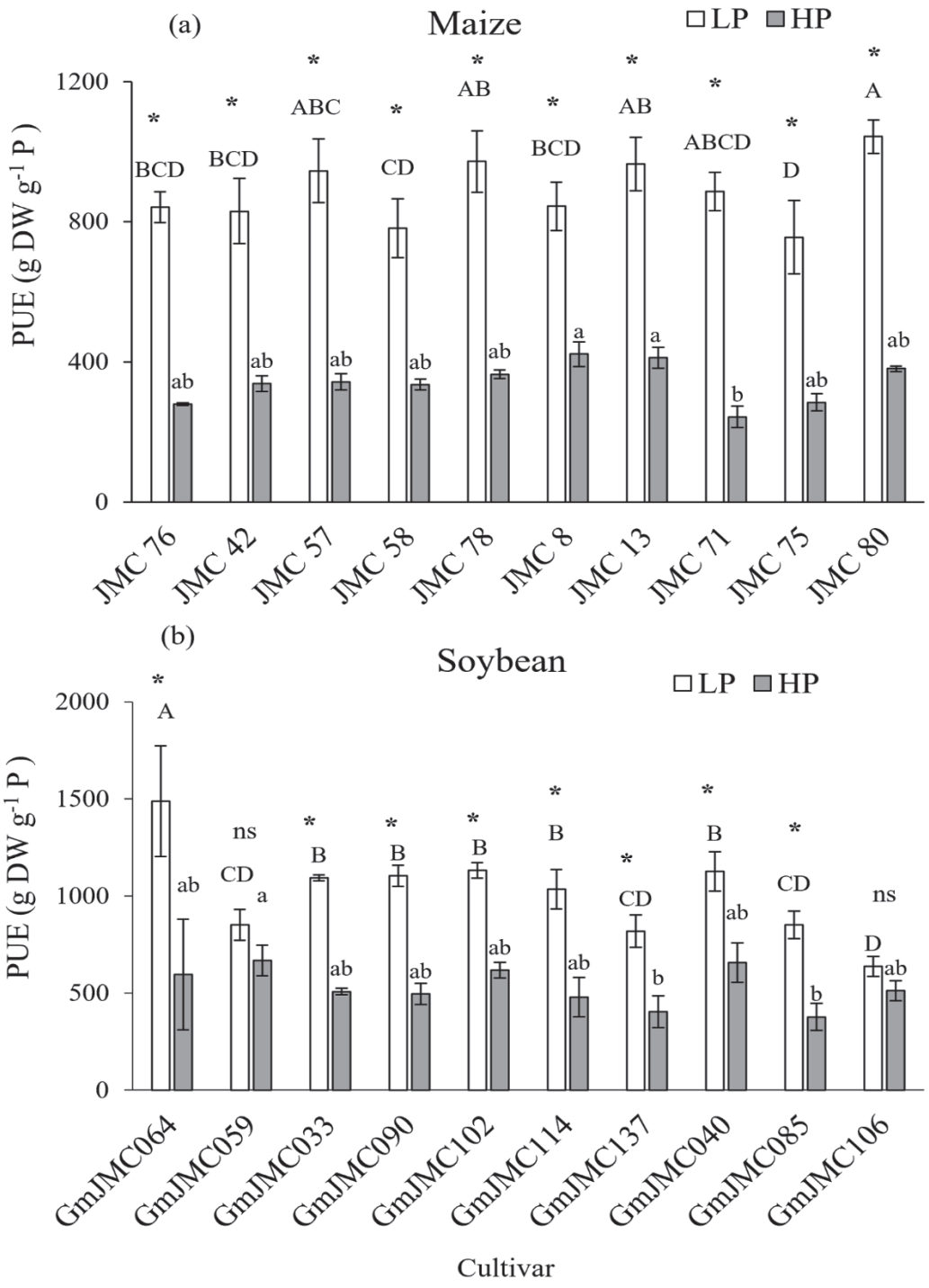

Figure 3.8 Phosphorus use efficiency (PUE) of maize (a) and soybean (b) cultivars under low and high P conditions. Values not followed by a common letter in each cultivar at each P supply differ significantly ($p < 0.05$). Error bars represent the standard error (S.E.; n = 4). Cultivars indicated with an asterisk are significantly different ($p < 0.05$) in between low P and high P; ns (not significant).

3.4.4. ACP activity and acidification of rhizosphere soil

The amelioration in ACP activity of rhizosphere soil of maize under low P condition seemed distinct in all cultivars (Figure 3.9(a)). It was significantly higher than the high P in all cultivars except JMC 58. Any differences in ACP activity among selected maize cultivars were different at P status. Under low P conditions, genotypic variation was observed in the ACP activity of rhizosphere soil. In soybean cultivars, the increase in acid phosphatase activity in the rhizosphere under low P seemed not prominent except in GmJMC114 and GmJMC040 (Figure 3.9(b)). In soybeans, genotypic variation could also be observed under low P conditions. Under low P, the ACP activity of soybean cultivars GmJMC114, GmJMC137, GmJMC040, GmJMC085 and GmJMC033 were not statistically different. The GmJMC085 resulted in higher values for the ACP under both P conditions. The ACP activity of rhizosphere soil of maize cultivars ranged from 512.6 to 1302.9 (μg NP g^{-1} DW h^{-1}) under low P. Soybean cultivars ranged from 276.6 to 591.4 (μg NP g^{-1} DW h^{-1}) under low P. This indicates that ACP activity under low P depends on plant species and compared to maize, soybean resulted in weak ACP activity in rhizosphere soil.

Almost all maize cultivars reduced rhizosphere pH under P deprivation compared to high P, but a significant decrease was observed only in cultivars JMC 76, JMC 42 and JMC 71 (Figure 3.10(a)). The cultivars JMC 76 and JMC 71 with the divergent shoot and root responses under low P had a characteristic decrease in rhizosphere pH under low P. The rhizosphere soil pH was significantly lower in soybean cultivars GmJMC059, GmJMC033, GmJMC114, GmJMC085 and GmJMC106 under low P conditions (Figure 3.10(b)). Therefore, compared to maize cultivars, the reduction in rhizosphere pH was noticeable among the soybean cultivars under low P. Further, genotypic variation in rhizosphere soil pH in soybeans was observed under low P conditions. Like maize, the soybean cultivars GmJMC085 and GmJMC106, with contrasting responses under low P, had significant acidification of rhizosphere soil under low P.

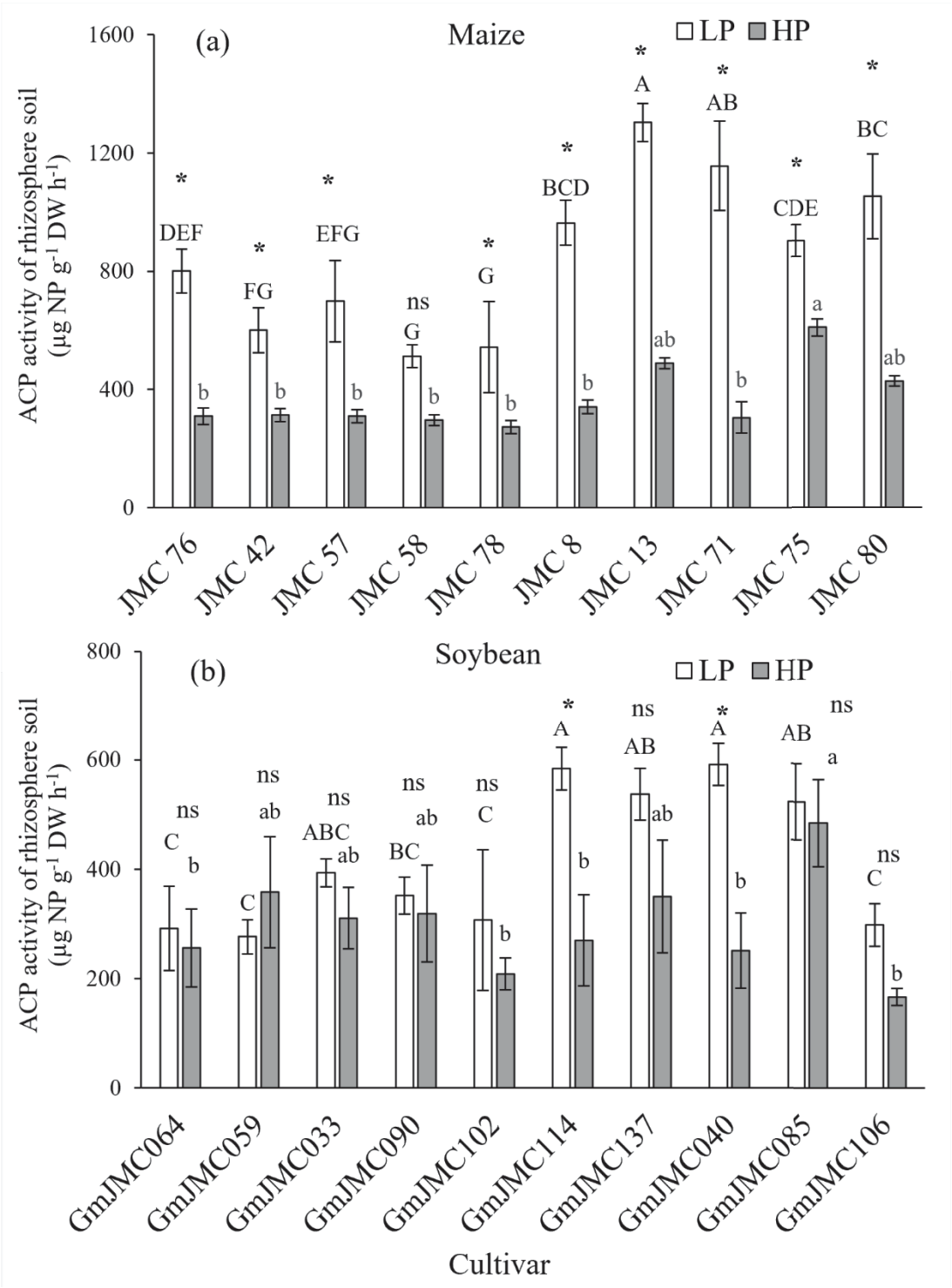

Figure 3.9 ACP activity of soil rhizosphere in maize (a) and soybean (b) cultivars under low and high P conditions. Values not followed by a common letter in each cultivar at each P supply differ significantly ($p < 0.05$). Error bars represent the standard error (S.E.; n = 4). Cultivars indicated with an asterisk are significantly different ($p < 0.05$) in between low P and high P; ns (not significant).

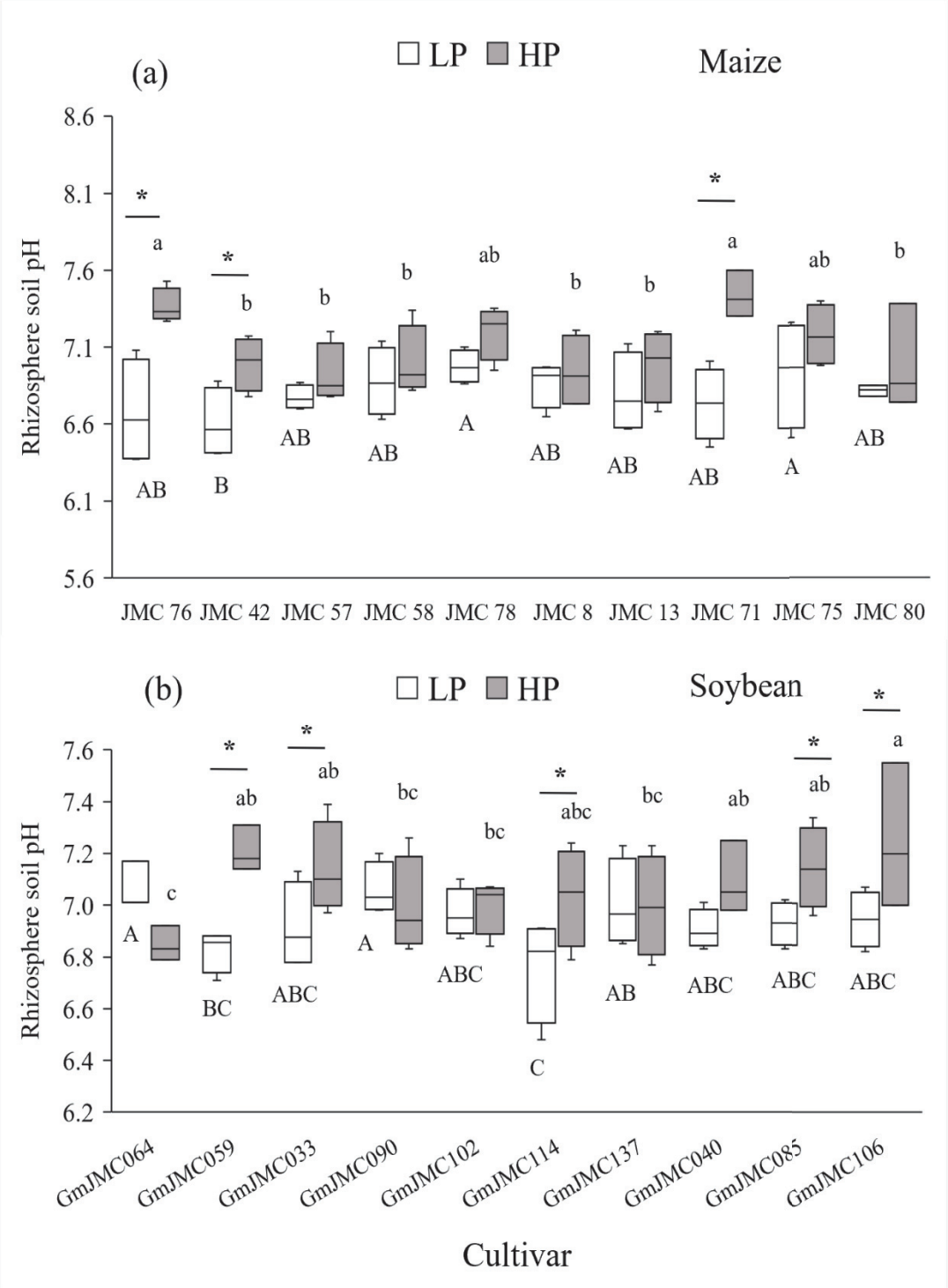

Figure 3.10 Changes of rhizosphere soil pH of maize (a) and soybean (b) cultivars under low and high P conditions. The box plots show the medians, 25th and 75th percentiles. The whiskers extend to 1.5 times the interquartile range. Values not followed by a common letter in each cultivar at each P supply differ significantly ($p < 0.05$). Cultivars indicated with an asterisk differ significantly ($p < 0.05$) between low P and high P.

3.4.5. Pearson's correlation and principal component analysis (PCA)

Pearson's correlation analysis for genotypes of maize and soybean showed that most traits had significant correlations under either P levels, except for ACP activity in maize and rhizosphere pH in soybean under high P conditions (Tables 3.5 and 3.6). For example, shoot dry weight significantly correlated with RDW and TRL and shoot P content at both P levels in both species (Tables 3.5 and 3.6). Under low P availability, the ACP activity of maize showed a significant negative correlation (R^2 = -0.52, $p < 0.001$) with shoot P content, indicating that low P availability will induce plant roots to release ACP to the soil. However, no association was found between ACP activity and shoot P concentration under low P conditions. Root P concentration of maize was strongly positively correlated with the ACP activity of rhizosphere soil under low P treatment. Contrary to maize, there was no correlation between ACP activity and shoot P content in soybeans under low P conditions (Table 3.6).

The first principal component (PC1) accounted for 42% of the total variation in maize under low P conditions (Figure 3.11(a)). PC1 had a strong correlation (either positive or negative) with root P concentration, ACP activity, shoot P content, SRL, SDW and TRL. PC2 accounted for 23% of the total variation and was mainly dominated by RRS, root P content, shoot P concentration, RDW and PUE. The cultivars JMC 58, JMC 76, and JMC 78 were mainly characterized by greater SDW and SRL and shoot P content under low P (Figure 3.11(a)). The acute angles between loading vectors indicated that SDW was strongly positively correlated with SRL, shoot P content and TRL under P deprivation. Under high P conditions, PC1 represented 57% of the total variation and was mainly dominated by RDW, PUE, SDW, TRL, shoot P concentration and rhizosphere pH (Figure 3.11(b)). PC2 contributed 20% of the total variation and was mainly influenced by SRL. Under high P, JMC 8 is mainly characterized by PUE, RDW, RRS and root P content. On the other hand, the cultivars JMC 78, JMC 58, JMC 57 and JMC 80 were mainly influenced by SDW, TRL and shoot P content under high P. Under both P regimes, cultivars JMC 57, JMC 78 and JMC 58 were characterized by similar variables (Figure 3.11(a and b)). Further, JMC 71 and JMC 13 considerably deviated from other genotypes under both P supplies.

In the low P treatment of soybean, PC1 and PC2 contributed 45% and 25% of the variation, respectively (Figure 3.11(c)). A strong negative correlation between PC1 could be observed in SDW, RRS, RDW, TRL and root P content. GmJMC085 stood for high values of SDW, RDW, TRL, RRS, shoot P content and ACP activity under low P. The acute angles between the loading vectors showed that SDW and RDW, TRL and ACP, and SRL and shoot P concentration strongly correlated. GmJMC114, GmJMC090, GmJMC040 and GmJMC033 clustered together, and the cultivars GmJMC059, GmJMC064 and GmJMC106 deviated from the rest (Figure 3.11(c)). Under the high P, PC1 represented 56% of the total variation, and shoot and root P contents, TRL and shoot P concentration mainly dominated it (Figure 3.11(d)). PC2 represented 20% of the total variation and was dominated by RRS, SRL and SDW. PUE and shoot and root P concentrations had strong negative relationships. Under sufficient P condition, GmJMC085 was characterized by ACP activity, SRL, shoot and root P concentrations and contents. The cultivars GmJMC059, GmJMC040 and GmJMC106 deviated from the rest of the cultivars under the high P (Figure 3.11(d)).

Table 3.6 Pearson's correlation analysis for maize genotypes under two P levels

	SDW	RDW	TRL	RRS	SRL	SPCon.	SPC	PUE	RPCon.	RPC	ACP	RpH
SDW		0.59***	0.67***	-0.28	0.59***	-0.03	0.84***	0.07	-0.61***	0.14	-0.48**	0.13
RDW	0.74***		0.85***	0.58***	0.39	-0.09	0.45**	0.09	-0.48**	0.66***	-0.11	-0.02
TRL	0.77***	0.80***		0.30	0.79***	0.05	0.59***	-0.06	-0.60***	0.39*	-0.42*	0.03
RRS	0.38*	0.88***	0.57***		-0.14	-0.07	-0.29	0.04	0.06	0.65***	0.38*	-0.09
SRL	0.00	-0.23	0.34	-0.36		0.21	0.60***	-0.23	-0.51**	0.02	-0.60***	0.04
SPCon.	-0.73***	-0.65***	-0.50**	-0.51**	0.30		0.50**	-0.98***	0.29	0.12	-0.15	0.40*
SPC	0.81***	0.49**	0.68***	0.18	0.21	-0.30		-0.46**	-0.39*	0.15	-0.52***	0.33*
PUE	0.72***	0.69***	0.47**	0.52***	-0.36	-0.93***	0.20		-0.25	-0.07	0.12	-0.35*
RPCon.	-0.46**	-0.35	-0.40*	-0.32	-0.07	0.45**	-0.39	-0.36		0.32	0.53***	0.19
RPC	0.41*	0.61***	0.52***	0.55***	-0.04	-0.38	0.22	0.41*	-0.14		0.37*	0.16
ACP	-0.07	0.14	-0.05	0.28	-0.32	0.01	-0.07	0.06	0.12	-0.16		-0.07
RpH	-0.60***	-0.62***	-0.52***	-0.44**	0.11	0.41*	-0.50**	-0.43*	0.06	-0.25	0.04	

The correlation coefficient of each trait under high P (lower left) and low P (upper right). Significant correlations are indicated by * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$). n = 40 (10 genotypes × 4 replicates) for every correlation. Traits included in this analysis are shoot dry weight (SDW), root dry weight (RDW), root-to-shoot ratio (RRS), total root length (TRL), specific root length (SRL), phosphorus use efficiency (PUE), shoot P concentration (SPCon.), root P concentration (RPCon.), shoot P content (SPC), root P content (RPC), ACP activity (ACP) and Rhizosphere pH (RpH).

Table 3.7 Pearson's correlation analysis for soybean genotypes under two P levels

	SDW	RDW	TRL	RRS	SRL	SPCon.	SPC	PUE	RPCon.	RPC	ACP	RpH
SDW		0.78***	0.74***	0.12	-0.17	-0.29	0.67***	0.16	-0.30	0.45**	0.12	0.00
RDW	0.69***		0.88***	0.70***	-0.31*	-0.41**	0.42**	0.24	-0.38*	0.54***	0.35*	-0.10
TRL	0.60***	0.71***		0.52***	0.12	-0.21	0.54***	0.07	-0.41**	0.36	0.38*	-0.05
RRS	-0.04	0.68***	0.35*		-0.41**	-0.41**	-0.13	0.27	-0.21	0.43**	0.44**	-0.15
SRL	0.27	0.17	0.81**	-0.06		0.57***	0.22	-0.45**	0.01	-0.37*	-0.03	0.09
SPCon.	-0.07	0.01	0.21	0.05	0.22		0.49**	-0.93***	0.03	-0.41**	-0.16	-0.07
SPC	0.42*	0.36*	0.51**	0.06	0.35*	0.87***		-0.55***	-0.28	0.10	0.01	-0.08
PUE	0.05	0.00	-0.20	-0.03	-0.20	-0.97***	-0.85***		-0.02	0.29	0.05	0.12
RPCon.	0.01	0.01	0.20	0.01	0.22	0.86***	0.76***	-0.82***		0.50**	0.07	-0.12
RPC	0.36*	0.54***	0.59***	0.37*	0.33*	0.76***	0.86***	-0.72***	0.83***		0.38*	-0.21
ACP	0.15	0.37*	0.40*	0.37*	0.24	0.03	0.09	0.01	-0.06	0.20		-0.33*
RpH	-0.15	0.09	0.09	0.21	0.04	0.12	0.08	-0.16	0.01	0.11	-0.10	

The correlation coefficient of each trait under high P (lower left) and low P (upper right). Significant correlations are indicated by * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$). n = 40 (10 genotypes × 4 replicates) for every correlation. Traits included in this analysis are shoot dry weight (SDW), root dry weight (RDW), root-to-shoot ratio (RRS), total root length (TRL), specific root length (SRL), phosphorus use efficiency (PUE), shoot P concentration (SPCon.), root P concentration (RPCon.), shoot P content (SPC), root P content (RPC), ACP activity (ACP) and Rhizosphere pH (RpH).

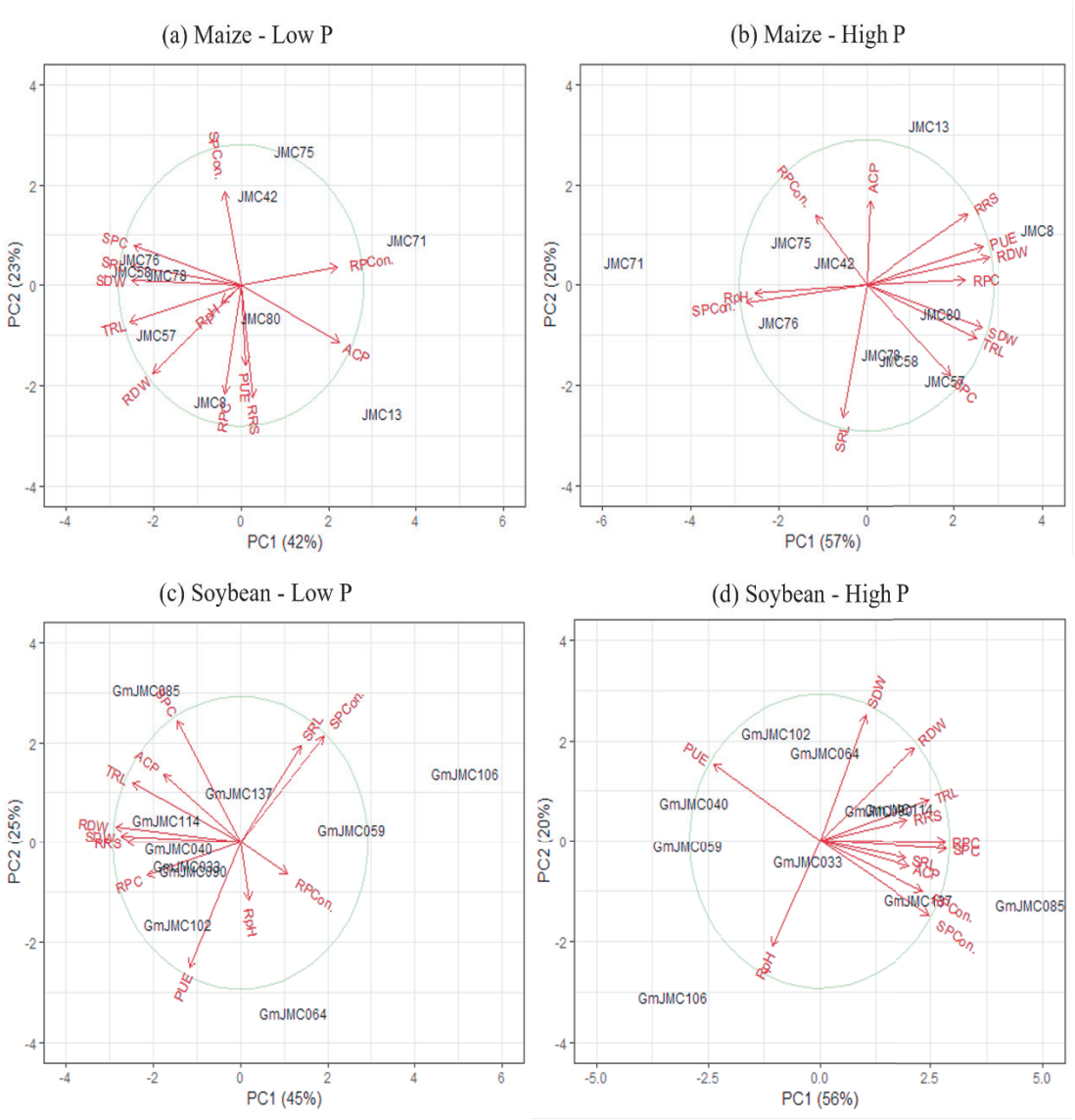

Figure 3.11 Principal component analysis for maize under low P (a) and high P (b) and soybean under low P (c) and high P (d) conditions. Vectors stand for trait factor loading coordinates for PC1 and PC2. Traits included in this analysis are shoot dry weight (SDW), root dry weight (RDW), root-to-shoot ratio (RRS), total root length (TRL), specific root length (SRL), phosphorus use efficiency (PUE), shoot P concentration (SPCon.), root P concentration (RPCon.), shoot P content (SPC), root P content (RPC) and ACP activity (ACP) and Rhizosphere pH (RpH).

3.4.6. Comparison of responses to P deficiency in maize and soybean with contrasting root morphology

3.4.6.1. Plant growth at different P availability

The interaction between species and P supply for SDW was significant, indicating that any difference between species on SDW differed at the P supply rate (Table 3.8). Low P caused a drastic reduction of SDW in both species, where the decline was 51% and 34% in maize and soybean, respectively. Even though SDWs were significantly different between maize and soybean under high P, they were not markedly different under low P (Figure 3.12(a)). RDWs were not notably different between the two species and P supply rates, emphasizing that more biomass was allocated to roots under low P conditions (Figure 3.12(b)). Under both P conditions, TRLs were significantly higher in maize than in soybeans. It was a 160% and 146% increase in high P and low P, respectively, compared to soybean. TRLs were not significantly different between high P and low P in both species (Figure 3.12(c)). RRSs were substantially higher in low P than high P, with an 86% and 71% increase in maize and soybean severally. Under both P regimes, soybean significantly increased the RRS compared to maize, with a 55% and 42% increase in high P and low P, respectively (Figure 3.12(d)). Low P stress did not cause a change in SRL in both species compared to high P, and it was significantly lower in soybean compared to maize in both P supply rates. Further, soybean was 63% and 68% lower in high P and low P, respectively, than maize (Figure 3.12(e)).

3.4.6.2. P status in shoots and roots and PUE

The interactions between species and P supply for shoot and root P concentrations and contents were significant, indicating that any differences between species on the above variables differed at the P supply rate (Table 3.8). P deficiency caused a drastic reduction in shoot and root P concentrations and contents (Figure 3.13). The decline in shoot P concentrations was 62% and 47% in maize and soybean, respectively, compared to high P. Even though shoot P concentrations were significantly different under high P conditions, they were not notably different under low P (Figure 3.13(a)). Shoot P concentration under high P was markedly higher in maize.

In contrast, root P concentration under high P was significantly higher in soybean roots (Figure 3.13(b)). Root P concentrations declined by 39% and 65% in maize

and soybeans. Similar trends could be seen in shoot and root P contents. Though high P caused a significant difference between maize and soybean, there was no significant difference for shoot and root P contents under low P (Figure 3.13(c and d)). Like shoot and root P concentrations, shoot P content was significantly higher in maize. Contrary to that, root P content was notably higher in soybeans (Figure 3.13).

Table 3.8 Significance of different sources of variance

Parameter	Source of variability		
	S	P	S × P
Shoot dry weight (g plant^{-1})	0.001	<0.001	0.01
Root dry weight (g plant^{-1})	0.151	0.930	0.160
Total root length (cm plant^{-1})	<0.001	0.630	0.949
Root-to-shoot ratio	<0.001	<0.001	0.379
Specific root length (m g^{-1})	<0.001	0.172	0.196
Shoot P concentration (mg g^{-1})	<0.001	<0.001	<0.001
Root P concentration (mg g^{-1})	0.482	<0.001	<0.001
Shoot P content (mg P plant^{-1})	<0.001	<0.001	<0.001
Root P content (mg P plant^{-1})	0.004	<0.001	0.005
Phosphorus use efficiency (g DW g^{-1} P)	<0.001	<0.001	0.458
Acid phosphatase activity (µg NP g^{-1} DW h^{-1})	<0.001	<0.001	0.001
Rhizosphere pH	0.229	<0.001	0.057

Probability values in the table are related to a two-way analysis of variance for the factors species (S), phosphorus supply (P), and the interaction of species × phosphorus supply (S × P) at the significance level of ($p < 0.05$).

P deficiency caused notable enhancement in phosphorus use efficiency in both species; it was a 160% and 91% increment in maize and soybean, respectively, compared to high P. Compared to maize, soybean enhanced its PUE by 56% and 14% under high P and low P, respectively. However, the increment under low P was not significantly different between the two species (Figure 3.13(e)).

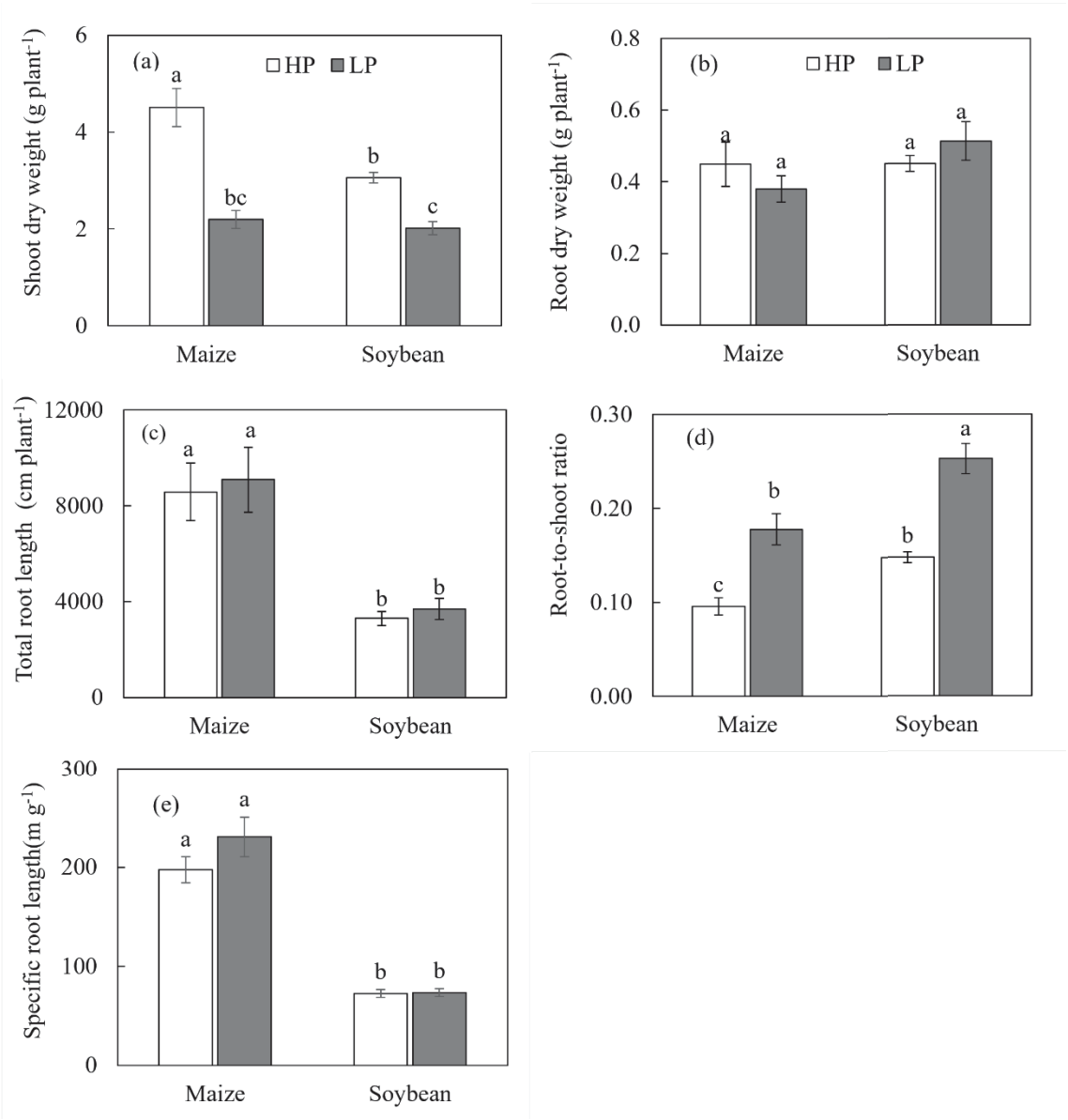

Figure 3.12 Plant growth at different P availability; SDW (a), RDW (b), TRL (c), RRS (d), and SRL (e) between maize and soybean under low P and high P conditions. Values not followed by a common letter differ significantly ($p < 0.05$). Error bars represent the standard error (S.E.; n = 10).

3.4.6.3. ACP activity and acidification of rhizosphere soil

P deficit condition ameliorated ACP activity in rhizosphere soil in both species (Figure 3.14(a)), but amelioration was only significant in maize compared to high P. Maize showed 132% more ACP activity under low P than high P. Contrary to that, soybean showed 40% enhancement, which was insignificant compared to a high P. Under P

deficiency, the ACP activity of maize in rhizosphere soil was two times higher than in the soybean. Under high P, the ACP activity of rhizosphere soil was not significantly different between the two species (Figure 3.14(a)).

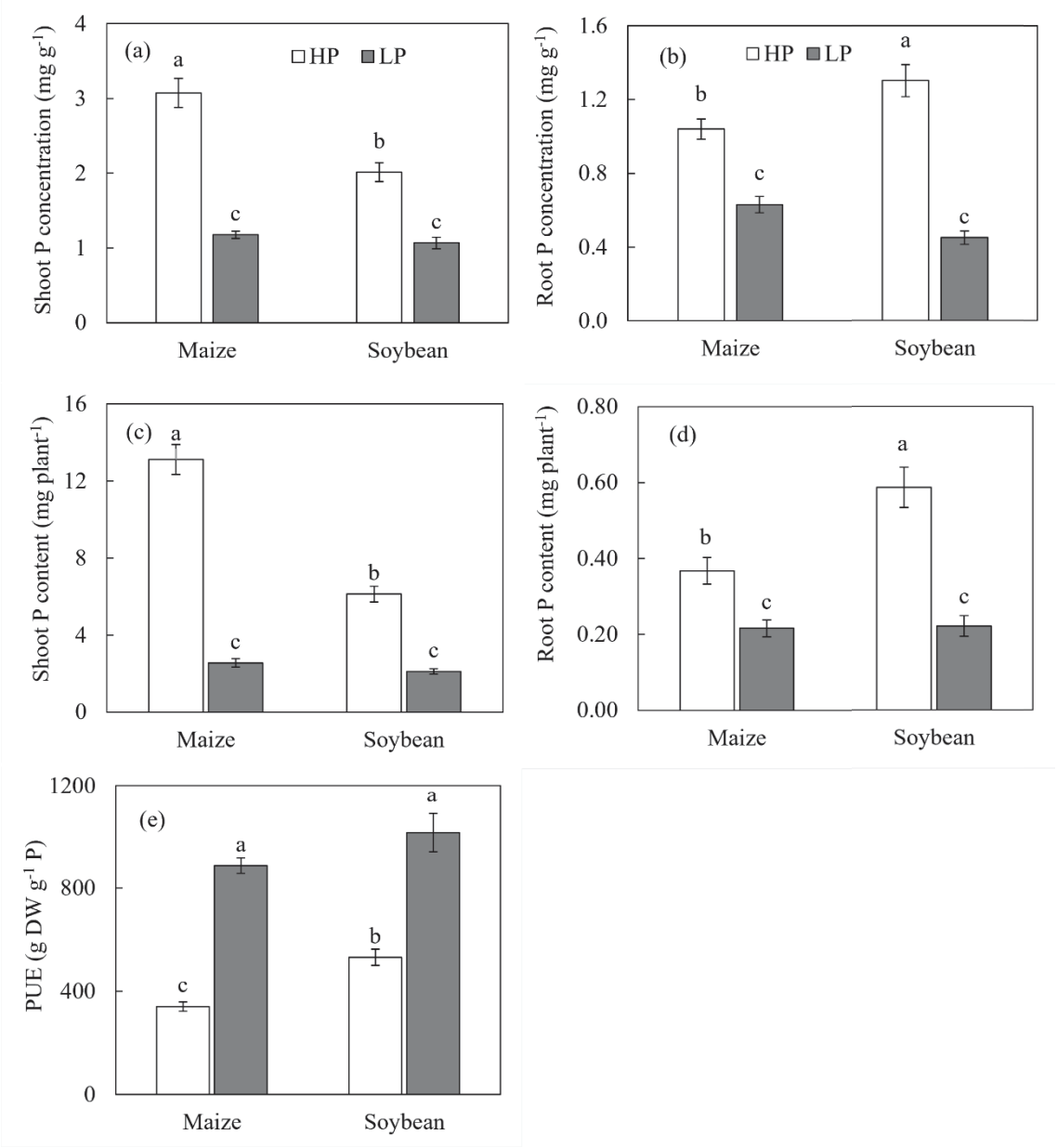

Figure 3.13 P accumulation and PUE of maize and soybean under low P and high P; shoot P concentration (a), root P concentration (b), shoot P content (c), root P content (d), and PUE (e). Values not followed by a common letter differ significantly ($p < 0.05$). Error bars represent the standard error (S.E.; n = 10).

Under low P, both species acidified their rhizosphere soil compared to high P (Figure 3.14(b)). Nevertheless, the pH of rhizosphere soil was significantly lower only in maize compared to high P. Under high P conditions, the pH of rhizosphere soil was not notably different between the two species (Figure 3.14(b)).

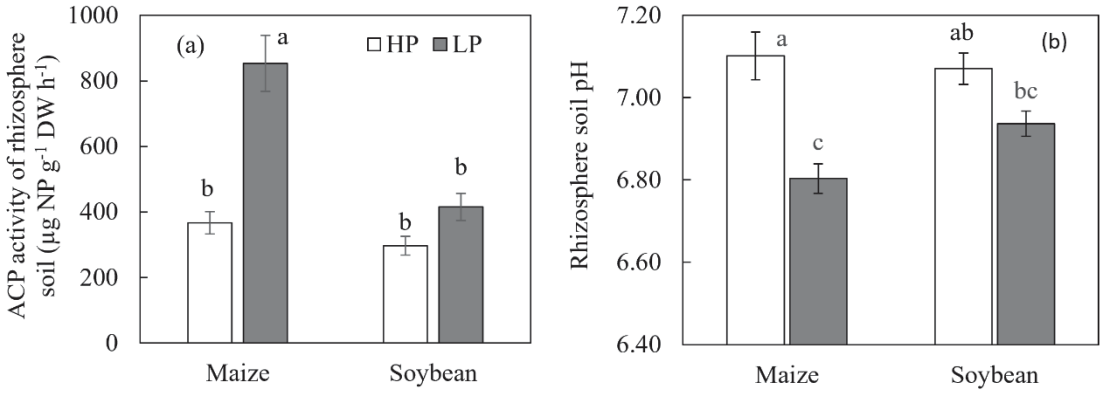

Figure 3.14 ACP activity and acidification of rhizosphere soil of maize and soybean under low P and high P; ACP activity of rhizosphere soil (a), rhizosphere soil pH (b). Values not followed by a common letter differ significantly ($p < 0.05$). Error bars represent the standard error (S.E.; n = 10).

3.5. Discussion

To understand the adaptive responses of shoot and root to low P in selected cultivars of the Japanese core collections of maize and soybean, we carried out hydroponic screening followed by a soil experiment using Regosols. This study aimed to understand the adaptive responses of shoot and roots, rhizosphere ACP activity and acidification, and their genotypic variability. Furthermore, the effects of plant-plant interactions were minimized in our study since we grew one plant per pot in the soil experiment.

Soil properties can significantly influence root developmental responses to P deficiency. Cong et al. (2020) proposed integrating four plant strategies targeting crop genotypes and prioritizing those strategies depending on the soil P status. Regosols is a weekly developed sandy soil characterized by low P absorption capacity (Table 3.4). Therefore, even heavy application of P fertilizers will no longer be retained in the Regosols and will cause water pollution through leaching and runoff. Consequently, high P acquisition strategies are critical in Regosols with both high/low plant-available

soil P. According to the results of the present study, the differences among the selected cultivars of both species in their ability to use P under low P and high P conditions indicate genotypic variability in their responses to P supply (Figures 3.1-3.5). Furthermore, identifying such genotype-specific root modification under low P availability in Regosols can be combined with agronomic strategies like intercropping to enhance overall P use efficiency by including crop species or genotypes characterized by strong P mobilizing or foraging capacity.

3.5.1. Variation in plant growth and root plasticity

To cope with P deficiency, plants have evolved a variety of morphological, physiological and biochemical responses (Dissanayaka et al., 2018; Zhang et al., 2014). Modification in root architecture is one of the critical strategies to enhance P uptake under low P stress. Previous studies have shown that under low P stress, most species allocate more biomass to roots, increase root length, and develop more lateral roots and dense root hairs to promote P uptake (Chen et al., 2018; Gaikpa et al., 2022). Our results also showed a pronounced increase in RRS across the cultivars tested in both maize and soybean (Figure 3.4). Under P deficiency, increased RRS is caused by decreased shoot growth and increased carbon allocation from the shoot to the roots (Hermans et al., 2006). However, we found differences in RDW, TRL and SRL among different cultivars in both species under low P (Figures 3.2, 3.3 and 3.5). In maize at low P, RDWs were either significantly decreased (JMC 8, JMC 13 and JMC 80) or not significantly different compared to high P (Figure 3.2(a)) among the tested cultivars. TRLs were either significantly increased (JMC 76), decreased (JMC 80) or not significantly different relative to high P (Figure 3.3(a)). Contrary to maize, soybean RDWs were almost significantly increased, and TRLs were either significantly increased (GmJMC040 and GmJMC085) or not significantly different compared to high P (Figure 3.2(b)). These results imply that different root responses to P deficiency are genotype-specific (Liu et al., 2018b).

Furthermore, the above responses of some of the cultivars of the two species were distinct under low P, indicating their low P tolerance. In maize, the cultivars JMC 57, JMC 76, JMC 8 and JMC 58, and in soybean, the cultivars GmJMC033, GmJMC040 and GmJMC085 showed distinct modification in root morphology, including high RRS, SRL, and TRL (Figures 3.1, 3.11(a and c) and 3.15) to produce

comparatively greater shoot biomass under low P by increasing P acquisition. If it is further investigated, the cultivars showed distinct modifications; there were not many differences among the maize cultivars JMC 57, JMC 76, JMC 8 and JMC 58 under low P in their modifications except in a few changes in RRS and SRL (Figures 3.1-3.5(a)). The maize cultivars JMC 76, JMC 8 and JMC 58 produced a similar amount of biomass with reduced metabolic cost of soil exploration compared to the cultivar JMC 57 (Figure 3.5(a)). In the case of low P-tolerant soybean cultivars: GmJMC033, GmJMC040 and GmJMC085, GmJMC085 showed remarkable responses with regards to RDW, TRL and SRL compared to the other two cultivars under low P conditions (Figures 3.2, 3.3 and 3.5(b)). Thus, soybean cultivar GmJMC085 produced greater biomass with reduced metabolic cost of soil exploration than soybean cultivar GmJMC033 (Figure 3.5(b)). Previous studies also suggested that preferential partitioning of assimilates to roots could capture more P by exploring large volumes of soil (Kvakić et al., 2020; Wissuwa et al., 2005). Besides, genotypes with reduced metabolic costs of soil exploration are imperative to improve P acquisition under low P stress (Long et al., 2019). In addition, the maize cultivars JMC 13, JMC 71, and soybean, the cultivars GmJMC064, GmJMC059 and GmJMC106 were P-inefficient due to poor modification in root growth (Figures 3.1-3.5, 3.11 and 3.15). Several other studies also proved that relative to P-inefficient genotypes, P-efficient genotypes often have more extensive root systems with greater biomass or density (Azevedo et al., 2015; Corrales et al., 2007; Zhou et al., 2016). Our results also highlighted that compared to low P-sensitive genotypes of both species, tolerant genotypes could modify their root system by increasing RRS, TRL and SRL for acquiring P to improve tolerance to P deficiency (Figures 3.1-3.5 and 3.11(a and c)). However, further research is needed to examine the mechanisms related to low P tolerance.

Genotypic variations are imperative for future breeding initiatives to produce more P-efficient genotypes. Among the cultivars with distinct root modifications, the maize cultivar JMC 76 and soybean cultivar GmJMC033 showed no significant differences in their SDW between low P and high P as low P-tolerant cultivars (Figure 3.1). Their relative shoot growths under low P stress compared to high P were 83% and 81%, respectively (Figure 3.16). The genotypes with more biomass at both P levels, such as soybean cultivar GmJMC033, are genuinely beneficial because they can be

fitted into a range of P levels without decreasing biomass (Bilal et al., 2018). However, the biggest concern in screening for P-efficient genotypes is producing more biomass under low P conditions (Bilal et al., 2018; Long et al., 2019).

Figure 3.15 Different root growth responses of soybean and maize cultivars grown in hydroponic conditions under low P stress during initial screening. Soybean cultivars: GmJMC033 (low P-tolerant) (a), GmJMC106 (low P-sensitive) (b), maize cultivars: JMC 57 (low P-tolerant) (c) and JMC 13 (low P-sensitive) (d).

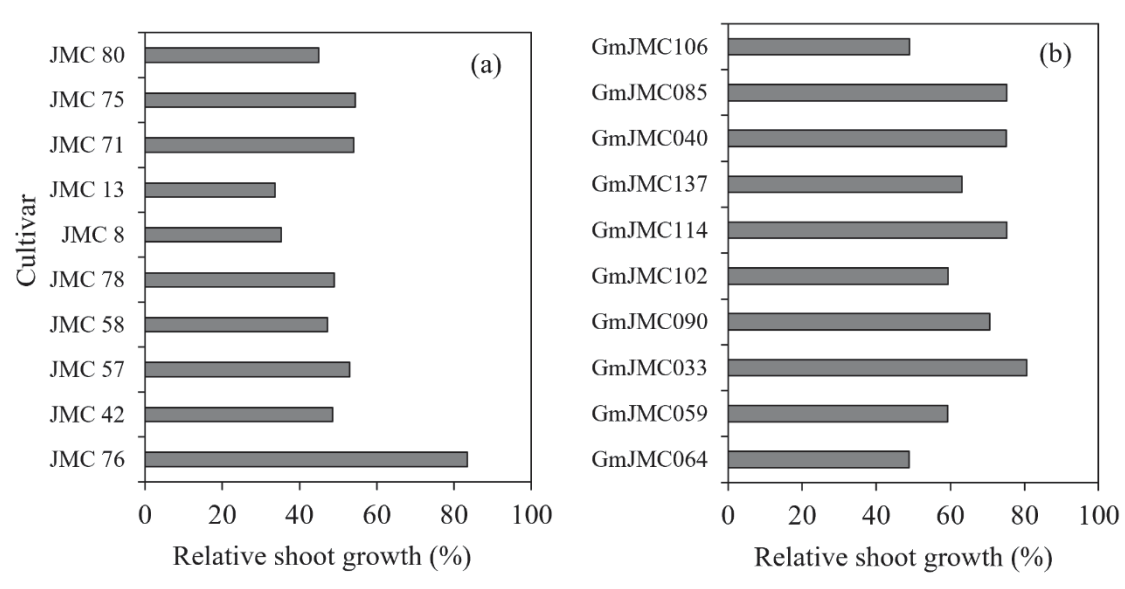

Figure 3.16 Shoot growth of maize (a) and soybean (b) genotypes grown under low P conditions relative to growth at high P conditions.

3.5.2. P accumulation and PUE

In this study, we found that the traits such as shoot and root biomass, root length, and shoot and root P contents were highly correlated in both species under low P availability (Tables 3.6 and 3.7). The positive correlations between root dry weight and shoot and root P contents confirm that genotypes with enhanced root growth under low P conditions could explore more nutrients. Further, PCA-derived results also supported the idea that low P-tolerant cultivars of both species had the maximum expression level of the above traits under low P conditions (Figure 3.11(a and c)). Further, a positive correlation between root length and shoot P content supports P uptake and translocation of P from root to shoot. The shoot P concentration was lower in P-tolerant cultivars than in P-sensitive cultivars, e.g., in maize cultivars JMC 57 and JMC 71 and the soybean cultivars GmJMC085 and GmJMC106 (Figure 3.6). Therefore, the genotypes with poor biomass and high tissue P were considered poor performers under low P conditions (Bhatta et al., 2021).

P accumulation and use efficiency are two distinct aspects of crops where P uptake refers to the exploratory and absorptive capacity to forage P from the soil. PUE explains the amount of total biomass or yield produced per unit of absorbed P. PUE can be achieved through recycling acquired P within the plants to maximize the growth and biomass under low P stress. Plants can achieve improved PUE through optimal distribution and redistribution of P to harvestable plant parts, allowing maximum growth and biomass allocation under low P. The mechanisms include the replacement of phospholipids with galactolipids and sulfolipids, P scavenging from endogenous nucleic acid pools and P remobilization from senescing organs (Dissanayaka et al., 2018; Veneklaas et al., 2012). We observed sufficient genotypic variation for PUE and an increase in PUE under low P compared to high P conditions in both species (Figure 3.8). It indicates that acquired P was more utilized under low P than high P conditions (Yao et al., 2007; Li et al., 2021). Therefore, mechanisms that improve PUE could be coupled with P-tolerant genotypes under low P stress.

3.5.3. ACP activity and acidification of rhizosphere soil

It is well-documented that P impoverishment enhances ACP secretion to root rhizosphere to hydrolyze organic P as an essential adaptive mechanism under low P conditions (Janes-Bassett et al., 2022; Ma et al., 2021a; Wu et al., 2021). It enables plants

to mobilize part of the P fixed in unavailable pools and make them available for plant uptake. According to the results, almost all cultivars of maize enhanced ACP activity distinctly, even P-sensitive genotypes JMC 13 and JMC 71 (Figure 3.9(a)). Du et al. (2016) also reported that root-secretory ACP activity was induced by P deficiency regardless of genotype; however, the low P-tolerant line responded more rapidly than the low P-sensitive line to P deficiency. Therefore, our results further confirmed that P deficiency induces ACP activity as an adaptive mechanism in maize regardless of genotype. However, Kuzyakov and Domanski (2000) suggested that soil microorganisms, an essential source of ACP, are also concentrated in the rhizosphere. Contrarily, some reports have emphasized that plant roots have a significant impact on the composition and function of the rhizosphere microbial community, and phosphatases in rhizosphere soil are mainly due to the secretion of plant roots rather than microbial community under low P stress (Kandeler et al., 2002; Wasaki et al., 2008). In the case of soybean, low P stress caused enhancement in ACP in almost all cultivars, but it was not distinct in P-sensitive cultivars: GmJMC064, GmJMC059 and GmJMC106 (Figure 3.9(b)). The soybean cultivars GmJMC040, GmJMC085 and GmJMC033 are among the cultivars that showed higher ACP activity under low P, indicating that low P-tolerant cultivars of soybean are characterized by high ACP activity in rhizosphere soil (Figures 3.9(b) and 3.11(c)). Maize had more excellent ACP activity than soybean under low P conditions (Wu et al., 2021). Our results showed a genotypic variation in secreting ACP in maize and soybean cultivars under P deficit conditions (Figure 3.9). These variations are imperative for future breeding ventures in producing P-efficient genotypes.

Rhizosphere acidification is a widespread response to P deficiency, particularly in dicotyledonous plants (Lei et al., 2015). Organic acids are secreted from plant roots as a physiological adaptation to low P stress. Acidification enhances the solubility of P in the rhizosphere and increases the P uptake of plants. Some P-tolerant and sensitive maize and soybean cultivars significantly reduced rhizosphere soil pH under low P (Figure 3.10). Among the low P-tolerant maize cultivars, only JMC 76 significantly reduced rhizosphere soil pH and among the low P-tolerant soybean cultivars, GmJMC033 and GmJMC085 significantly dropped down the rhizosphere pH under

low P conditions (Figure 3.10). However, further research is needed to investigate organic acid exudation and its composition in different cultivars of maize and soybeans.

Further, our results highlighted that maize cultivars showed low P tolerance; JMC 76, JMC 57, JMC 58 and JMC 8 highly depended on root morphological traits rather than physiological traits (Figure 3.11(a)). Wen et al. (2017) and Lyu et al. (2016) also highlighted that maize depends on root morphology rather than physiological traits in response to P deficiency. Further, the soybean cultivar GmJMC085, characterized by well-defined morphological and physiological responses under P deficiency (Figure 3.11(c)), indicated that low P tolerance was due to both responses in soybean (Lyu et al., 2016).

3.5.4. Comparison of responses to P deficiency in maize and soybean with contrasting root morphology

P stressed plants in both species, drastically reduced shoot biomass, increased RRS and maintained root growth (Figure 3.12). These results are consistent with the results of Fernandez and Rubio (2015), Gaume et al. (2001) and Mollier and Pellerin (1999). The drastic reduction in shoot growth may be due to reduced leaf growth, and it can be assumed that the leaf demand for carbohydrates is reduced, and more carbohydrates are available for root growth. It may explain the slight stimulation of root growth under P deprivation (not statistically different with high P) in both species (Figure 3.12). The RRS was remarkedly increased on P-deprived plants in both species, indicating that shoot growth was more severely reduced than root growth (Figure 3.12). Previous studies also generally agreed that P deficiency leads to higher RRS (Fernandez and Rubio, 2015; Tang et al., 2020) and was associated with a higher carbohydrate content in the roots (Khamis et al., 1990). However, root response to P deficiency (increase or decrease) depends on the time scale and even though short-term P deficiency leads to maintained or slightly enhanced root growth, long-term P deficiency causes a reduction in root growth because of the reduced leaf area of P deprived plants severely reduces their capacity to intercept light (Mollier and Pellerin, 1999).

Though root biomass was not significantly different between maize and soybean under low P conditions, TRL and SRL were substantially higher in maize than in soybean, indicating that contrasting architectural strategies involved P acquisition

under P deficiency (Figure 3.12). Maize has a fibrous root system that is usually more deeply distributed than the basal roots of the tap root system of soybean (Niu et al., 2013). Further, the higher SRL values under low P stress indicate that the root system has a lower metabolic demand per unit of root length (Fernandez and Rubio, 2015). Maximizing soil exploration of P at the minimum metabolic cost is crucial under low P stress. Several root traits like root hair production, SRL, root cortical aerenchyma formation, and roots' distribution between diameter classes may affect the metabolic cost of soil exploration of P.

It has been reported that aerenchyma formation in P stressed maize and soybean roots (Fan et al., 2003; Fernandez and Rubio, 2015). The formation of root cortical aerenchyma reduces the maintenance of root respiration by replacing carbon-demanding cortical cells with air spaces. Further, it can reduce the P requirement of root growth under P-limited conditions (Fan et al., 2003). It further highlighted our root P concentration results, where root P concentrations were significantly lower in both maize and soybean under low P conditions than in high P, even though root biomass was not significantly different from high P (Figure 3.13). However, it can be assumed that the benefit of root cortical aerenchyma under P deprivation is larger for maize than soybean, where the root system is characterized by finer roots, which is more favorable for the P acquisition (Postma and Lynch, 2011).

Secondly, the proliferation of root hairs in maize at low P, particularly dense root hairs of the main axis and first-order nodal laterals, may be the reason for the significant difference between maize and soybean regarding TRL and SRL. An increase in root hair density and length in response to low P is a well-discussed fact in plant biology (Péret et al., 2011) because the plasticity response of root hairs is relatively faster than root growth and branching (Niu et al., 2013). Additionally, root hairs can change the absorptive surface area of the root to absorb available P before lateral root branching (Niu et al., 2013). Further, genetic variation in root hair length and density is a well-known fact in maize, and it has been suggested as an appropriate target for marker-aided selection to improve the P efficiency in maize (Zhu et al., 2005a). The most important fact is that root hair formation increases phosphorus acquisition at minimal carbon cost. Therefore, the SRL of root hairs is more remarkable than other classes of roots.

The third reason for the difference related to TRL and SRL between maize and soybean could be enhanced or sustained lateral rooting under low P availability in maize. Zhu and Lynch (2004) reported that maize genotypes with enhanced or sustained lateral rooting had greater P acquisition and biomass accumulation under low P deficiency and observed significant genotypic variation for lateral rooting under low P stress. Further, the cost-benefit analysis of lateral root development was advantageous under low P availability. Additionally, enhanced or sustained lateral rooting under low P generally had the largest SRL (Zhu and Lynch, 2004), which aligns with our study's results (Figure 3.12). The ability of lateral rooting with the reduced metabolic cost is associated with a smaller diameter and greater SRL than non-lateral root types. Fernandez and Rubio (2015) found that P stress caused a reduction in the root diameter of maize but not in soybean and sunflower. Further, low P levels increased the proportion of fine roots of maize, concentrating more than 50% of their root length in the fine root class. However, soybean has high P uptake efficiency supported by the fineness of the root system.

Generally, legumes are considered to have high P requirements due to symbiotic N fixation. Therefore, N-fixing root nodules of legumes are regarded as strong P sinks (Zhong et al., 2023), which coincides with the results of our study where root P concentrations are significantly higher in soybean than maize under high P conditions (Figure 3.13). Therefore, low P stress can severely impact the nitrogenase activity of nodules in legumes (Lu et al., 2020). Under low P stress, both species significantly improved PUE as an adaptive mechanism to withstand low P availability (Figure 3.13). Soybean resulted in higher PUE under both P conditions than maize, even though it was insignificant under low P stress (Figure 3.13). Under P deficiency, strategies enhancing P recycling, scavenging, and remobilization could increase PUE. Sulieman et al. (2013) reported that legumes under low P stress could remobilize a significant quantity of P from the shoot and root. Further, Okazaki et al. (2017) revealed that lipid remodeling was very active in older soybean leaves, and P was preferentially remobilized in younger tissues under low P stress.

Under low P, a remarkable difference in ACP activity between maize and soybean resulted (Figure 3.14). The physiological and biochemical alterations of root traits, like the exudation of organic acids, protons and acid phosphatases under P

deficiency, facilitate the mobilization of sparingly available P in the rhizosphere. It was further emphasized in the results of rhizosphere acidification, where both species reduced the rhizosphere pH under low P stress compared to high P (Figure 4.3). However, Wu et al. (2021) observed no significant differences in ACP activity between maize and soybean under low P and optimal P treatments. Moreover, Yun and Kaeppler (2001) suggested that ACP activity in maize might not be a major mechanism of scavenging or acquiring P under P starvation. Therefore, these findings further emphasized that significant variation exists among species and cultivars concerning physiological and biochemical alterations of root traits under low P stress. Thus, our samples contain considerable genotypic variation within the same species, and a general conclusion can be made based on the physiological characteristics under low P stress.

3.6. Conclusions

We observed significant genotypic variation in selected cultivars of Japanese core collections of maize and soybean under low and sufficient P conditions. The results showed that the plasticity of the root system characterized by high RRS, SRL and TRL contributes to the differences among genotypes. Their distinct modifications in morphological and physiological traits are crucial in considering future breeding ventures to produce more P-efficient crop genotypes. Among the tested maize cultivars, JMC 76, JMC 57, JMC 58 and JMC 8 and under the tested soybean cultivars GmJMC033, GmJMC040, and GmJMC085 showed distinctive root modification under low P stress, showing their low P tolerance. Further, identifying their quantitative trait loci (QTLs) would be more beneficial in understanding the genetic basis for their adaptations under low P stress. These genotypic adaptations could be combined with agronomic strategies to enhance the overall P use efficiency in diversified cropping systems like intercropping. Therefore, in future studies, we expect to investigate the plant-plant interactions as an inter-varietal study in a cereal-legume intercropping system under low P availability.

Compared to soybean, maize is responsive to P while increasing biomass accumulation under high P conditions. However, maize drastically reduced shoot growth under low P stress compared to high P than soybean reduced under low P. High RRS characterized both species, sustained TRL and SRL under low P stress, where maize

had significantly higher TRL and SRL compared to soybean, indicating a contrasting alteration in root traits. Nevertheless, both species prioritized enhanced PAE under low P stress. Low P availability significantly reduced the root P concentration of soybean compared to the reduction made by maize, indicating that biological N fixation could be severely impacted in legumes under low P stress. P-stressed maize and soybean enhanced PUE significantly, where soybean's PUE was higher than maize's under low P conditions. It implies that soybean can undergo P scavenging, recycling, and remobilization than maize under P deficiency. Modification of rhizosphere soil of both species resulted in enhanced ACP activity and a drop of rhizosphere soil pH under low P stress, which is favorable for mobilization of sparingly available P. ACP activity of rhizosphere soil was notably higher in maize than soybean under low P stress. Therefore, the complementary root traits under low P stress would be advantageous under a maize-soybean intercropping system.

CHAPTER 4

GENERAL DISCUSSION

4.1. Introduction

Phosphorus is an essential macronutrient that plays a central role in energy transfer and metabolism while also serving as a critical structural component of essential biomolecules such as phospholipids and nucleic acids (Brembu et al., 2017). However, Pi is one of the least available macronutrients in many agricultural soils worldwide. The over-application of Pi fertilizers is a common strategy to overcome P deficiency. It causes agricultural Pi runoff, a primary factor for disastrous environmental effects. Therefore, innovative approaches are required to decrease the excessive dependence of agriculture on depleting P resources. It could be achieved by manipulating crops to enhance their PAE or PUE (Dissanayaka et al., 2021; Heuer et al., 2017). Therefore, the exploitation of genetic variation in P acquisition, internal P utilization, and low total P and phytate P concentrations in seeds will have profound implications on the P cycle (Cong et al., 2020). Such an improvement is needed to manage limited phosphorus reserves and to reduce the disastrous environmental effects caused by the over-application of P fertilizers.

4.2. P acquisition

Available P uptake occurs through the collaborative action of several Pi transporters. P acquisition is greatly influenced by the root explorative and mining capacities, which are determined mainly by RSA, and the potential symbiotic association of the root with soil microbes, such as AMF, that aid the plant in scavenging P from the soil (López-Arredondo et al., 2014). Further, PAE is affected by other root traits that increase P availability from sparingly soluble P sources in the soil solution, including the exudation of organic acids, protons and phosphatases from the root into the rhizosphere.

There is considerable genotypic variation in P acquisition strategies for several crops (Lyu et al., 2016; Wang et al., 2016), including maize and soybean, as described in chapters 2 and 3. Also, plant species differ substantially in P acquisition strategies under low P stress (Lambers et al., 2015, chapter 3). For example, common bean and maize exhibit a genotypic variation in adventitious and lateral rooting and root hair development (Zhu and Lynch, 2004), while RSA varies substantially among soybean genotypes, indicating soybean genotypes with shallow root system is more P-efficient than genotypes with the deep root system (Zhao et al., 2004). Furthermore, chickpea

(*Cicer arietinum*) accessions differ substantially in the concentration of rhizosheath carboxylates (Pang et al., 2018). However, these different P acquisition strategies are associated with various metabolic and ecological costs (Lynch and Ho, 2005).

4.2.1. Pi transporters

Plants have high-affinity and low-affinity Pi uptake systems. The high-affinity system is functional at low P concentrations, whereas the low-affinity system operates at high P availability (Raghothama and Karthikeyan, 2005). The phosphate transporter (PHT) genes that encode Pi transporters have been isolated in Arabidopsis and grouped into four families: PHT1, PHT2, PHT3 and PHT4 (Raghothama and Karthikeyan, 2005). Significant progress has been made by characterizing Pi transporters in several important crop species, including rice, barley, maize, soybean, tomato and potato (*Solanum tuberosum*) (Fan et al., 2013; Glassop et al., 2007; Nagy et al., 2006; Nagy et al., 2005; Rae et al., 2003). Most research has focused on PHT1/PT, a high-affinity PHT family (Gu et al., 2016a). The soybean genome contains 14 PHT1/PT members that encode high-affinity Pi transporters, predominantly expressed in roots under low P stress (Fan et al., 2013). In maize, five PHT1 genes (ZmPht1;1-5) are induced under low P conditions in roots and in young and old leaves, anthers, pollen, and seeds (Nagy et al., 2006). Therefore, some members of the PHT1 gene family play an essential role in Pi uptake from the soil, particularly under P-limited conditions.

In contrast to the PHT1 family, members of the PHT2, PHT 3, and PHT 4 gene families have been associated mainly with Pi distribution within subcellular organelles, and specifically, they are in the plastid inner membrane, mitochondrial inner membrane, and Golgi body (Guo et al., 2008; Versaw and Harrison, 2002). For example, mitochondrial Pi transporter genes have been identified in soybeans, maize, and rice (Takabatake et al., 1999). Therefore, the expression of different Pi transporters is pivotal for efficient Pi uptake from the soil and the transport of Pi from the root to the shoot. Low P-tolerant genotypes highly express Pi transporter genes under P-limited conditions compared to low P-sensitive (Huang et al., 2011). As a result, exploiting the genetic diversity of major crops for alleles of high-affinity Pi transporters that improve P uptake efficiency is a feasible approach for breeding programs to enhance PAE.

4.3. Internal P use efficiency

Plants remobilize internal P, which is essential when soil P supply is limited. Internal P utilization efficiency is facilitated by transporting P from senescing organs to young ones, replacement of membrane phospholipids by galactolipids and sulfolipids that do not contain P, and scavenging P from internal organelles through scavenging hydrolases such as PAPs, phosphodiesterases, and ribonucleases (Bariola et al., 1999; Yu et al., 2002). These responses represent typical alterations occurring under P deficiency, and there is no clear indication that genotypic variation for such traits exists that could be exploited. Therefore, genotypic variation for PAE appears to be higher than PUE, and all advances in breeding P-efficient genotypes involve the exploitation of PAE traits (Parentoni and De Souza Júnior, 2008; Wissuwa et al., 2009). The mapping of several QTLs for PUE in various crops has been reported (Chen et al., 2009; Wissuwa et al., 1998; Yang et al., 2011; Zhang et al., 2017). Wang et al. (2009) overexpressed an Arabidopsis PAP gene (AtPAP15) in soybean. They reported higher yields in the transgenic plants when grown under low P conditions and concluded that AtPAP15 improved internal PUE in soybean transgenic plants.

Primarily, PUE had not been clearly defined. PUE is defined as biomass per unit of P. However, biomass may refer to entire plant biomass or yield, further unit of P may refer to P taken up by the plant, P applied to soil as fertilizers, or specific tissue P content (Rose and Wissuwa, 2012). Additionally, mechanisms related to higher PUE in genotypes of the same species are not well-known. Therefore, further research also needs to work on identifying valid QTLs from appropriate screening studies to determine the genes and mechanisms that confer enhanced genotypic PUE. Rose et al. (2011) suggested that QTLs for internal PUE should be determined under conditions in which PAE is equal for the cultivars studied, and Veneklaas et al. (2012) indicated that calculation of internal PUE indices based on the metabolically active P pools might provide a better insight to the molecular mechanisms related to PUE. Therefore, these strategies might aid in identifying valid QTLs and genes specific to internal PUE in crops (Veneklaas et al., 2012).

4.4. Photosynthetic P use efficiency (PPUE)

Plant productivity depends on photosynthesis, and photosynthesis relies on P-containing molecules. Therefore, efficient use of P in photosynthesis is a potentially crucial determinant of the PUE of crops. PPUE is the photosynthesis rate per unit leaf P (Veneklaas et al., 2012). The highest PPUE tends to be achieved by leaves with high rates of photosynthesis, high P concentration and low leaf mass per area (LMA) in fast-growing annuals, including annual crops (Veneklaas et al., 2012). Further, PPUE can vary by order of magnitude at any LMA, and part of this variation is attributed to leaf N concentration (Reich et al., 2009). Therefore, elucidation of variation in PPUE among and within crop species may be worthwhile and may give insights into mechanisms underlying high PPUE.

4.5. Genotypic variation for seed total and phytate P concentrations

Phytate, a mixture of salts of phytic acid, is the P-storage form in seeds, typically representing more than 75% of the total P of the seed (Raboy, 2003). Due to its strong chelating characteristic, phytate reduces the bioavailability of essential minerals such as calcium, manganese, magnesium, zinc, and iron, which may cause nutritional deficiencies in populations of developing countries. In addition, humans and non-ruminant animals (e.g., pigs and chickens) cannot efficiently metabolize phytate, which causes P losses to the environment.

As Cong et al. (2020) reported, there have been three main approaches to decreasing phytate concentrations in seeds. There are classical and molecular approaches to disturb phytate synthesis during seed development, molecular techniques to reduce P transport to seed, and classical genotypic exploitations to select cultivars with low phytate concentrations. Phytate synthesis can be disrupted through low phytic acid mutants. It involves the creation of gene knockout mutants by knocking out genes involved in the phytic acid biosynthesis pathway (Yamaji et al., 2017). Furthermore, the genetic modification approach can be used efficiently to reduce phytic acid content in cereals by cloning the genes of phytase enzymes. It creates the transgenic rice plant with a modified genome encoding for phytase enzyme to improve rice iron bioavailability to humans (Gupta et al., 2015).

Cong et al. (2020) investigated the variation in total seed P and phytate P concentrations among main grain cereals and legume genotypes. According to the results, soybeans exhibited the highest total seed P and phytate P concentrations among reviewed crops, and chickpeas showed the lowest phytate P concentrations in seed. Further, genotypic variation for seed phytate P concentration was more remarkable in soybeans, indicating the insights for exploiting genotypes with low phytate P concentration. Additionally, the authors emphasized that modern breeding programs should consider not only high yield but also low phytate concentrations.

4.6. Concluding remarks and future perspectives

Exploiting P-efficient genotypes is a promising way to conserve nonrenewable P resources, enhancing agricultural productivity and food security while mitigating adverse environmental impacts. Therefore, it is crucial to combine crop genotypes varying in PAE, internal PUE, PPUE and low seed phytate P concentration with agronomic strategies to enhance the overall PUE of cropping systems. High P acquisition strategies are critical in soils with sufficient and insufficient P availability through root foraging or mining capacities. Importantly, results presented in this dissertation indicate significant genotypic variation exists under both P-sufficient and P-limited conditions, thus suggesting potential for genetic improvement for P uptake and PUE for sufficient and limited P availability conditions.

Still, little is known about the mechanisms involved in the efficient genotypes of the same species under P starvation. Therefore, cultivars with contrasting P uptake and PUE in this study are also valuable for comparative physiological studies exploring the mechanistic differences resulting in their contrasting responses. Further, QTL analysis for efficient P acquisition for the identified cultivars of maize and soybean can be initiated in short order. Follow-up research should also consider organic acid exudation and AMF colonization under sufficient and insufficient P conditions. Finally, exploring the identified cultivars combined with agronomic strategies like intercropping systems will be essential. Therefore, in a follow-up study, selected P-efficient and inefficient cultivars of maize and soybean from Chapter 3 can be combined in a cereal-legume intercropping system to explore their facilitative interaction under low P stress.

Summary

Different shoot and root responses to low phosphorus availability in Japanese cultivars of maize and soybean

Introduction:

Low phosphorus (P) availability in agricultural soils severely impacts crop productivity worldwide. Over-applicating P fertilizers is not a viable solution to overcome P deficiency because such P is a non-renewable resource. Plants have evolved morphological, physiological, and biochemical responses to P deficiency. However, these morphological, physiological, and biochemical responses to P deficiency are species- and genotype-specific. Therefore, assessing the genotypic variability of crop genotypes under low P conditions and developing P-efficient crop genotypes are crucial to keeping the momentum of sustainable agriculture. Phosphorus efficient genotypes are advanced in either P acquisition efficiency (PAE) or P use efficiency (PUE). Strategies related to PAE and PUE are equally essential to improve the P efficiency of crops. Maize (*Zea mays* L.) and soybean (*Glycine max* L.) are important food crops with divergent root traits. Maize, a typical monocot, has a fibrous root system, whereas soybean, a typical dicot, is a tap-root crop. These crop species are essential in diversified cropping systems like intercropping or rotations. Accordingly, genotype strategies could be combined with agronomic strategies to enhance P efficiency jointly in the cropping system.

Research Objectives:

The study aimed to evaluate (1) genotypic variability of Japanese core collections of maize and soybean in response to low P availability, (2) different shoot and root responses of selected Japanese cultivars of maize and soybean, (3) acid phosphatase (ACP) activity and rhizosphere acidification of selected Japanese cultivars of maize and soybean, and (4) to compare different shoot and root responses to low P availability between two species.

Research Methodology:

The study comprised two preliminary screenings of Japanese core collections of maize (86 cultivars) and soybean (94 cultivars) under low P in hydroponic conditions and a pot experiment with Regosols for 30 days. During preliminary screening, soybean and maize cultivars were exposed to low P (50 μM) and (2 μM), respectively. Based on preliminary screening results, ten cultivars of each species were selected for further evaluation under soil conditions. The pot experiment had two P supply rates: low P (10 mg P kg^{-1} dry soil) and high P (50 mg P kg^{-1} dry soil). At harvest in both experiments, the shoot and roots were separated. Shoot dry weight (SDW), root dry weight (RDW), total root length (TRL), root-to-shoot ratio (RRS), and specific root length (SRL) were evaluated. All dried shoot and root samples were ground and digested for P determination using the HNO_3 and H_2O_2 digestion methods. In addition, at the harvest of the pot experiment, root systems were carefully lifted out of the soil with minimal damage, and rhizosphere soil samples were collected to evaluate ACP activity and rhizosphere acidification. PUE (dry weight per unit P uptake) was calculated as SDW divided by shoot P content.

Results and Discussion:

Genotypic variability of core collections of maize and soybean in response to low P:

Based on the preliminary screening results, maize and soybean core collections were clustered into 4 groups. Cluster analysis of soybean and maize revealed that soybean cluster III and maize cluster II characterized the highest Cluster mean for SDW, RDW, and TRL, indicating the availability of promising genotypes for the performance under low P stress in these two cluster groups. This study found that traits such as shoot and root biomass, root length, and shoot and root P contents were highly correlated under low P availability. The positive correlation between root dry weight and shoot and root P contents confirmed that genotypes with enhanced root growth under low P conditions could explore more nutrients.

Plasticity of the shoot and root growth responses:

Among the tested cultivars, except the maize cultivar JMC 76 and soybean cultivar GmJMC033, all other cultivars reduced the shoot biomass drastically under low P

stress. Their relative shoot growths under low P stress compared to high P were 83% and 81%, respectively. P deficiency caused an increase in RRS in almost all cultivars due to decreased shoot growth and increased carbon allocation from the shoot to the roots. However, we found differences in RDW, TRL, and SRL among different cultivars in both species under low P. In maize at low P, RDWs were either significantly decreased (JMC 8, JMC 13 and JMC 80) or not significantly different compared to high P among the tested cultivars. TRLs were either significantly increased (JMC 76), decreased (JMC 80) or not significantly different relative to high P.

Contrary to maize, soybean RDWs were almost significantly increased, and TRLs were either significantly increased (GmJMC040 and GmJMC085) or not significantly different compared to high P. These results imply that different root responses to P deficiency are genotype-specific. Furthermore, the above responses of some of the cultivars of the two species were distinct under low P, indicating their low P tolerance. In maize, the cultivars JMC 57, JMC 76, JMC 8 and JMC 58, and in soybean, the cultivars GmJMC033, GmJMC040 and GmJMC085 showed distinct modifications in root morphology, including high RRS, SRL, and TRL to produce comparatively greater shoot biomass under low P by increasing P acquisition.

A few differences existed among the low P-tolerant maize cultivars: JMC 57, JMC 76, JMC 8, and JMC 58 under low P in their modifications, especially in RRS and SRL. The maize cultivars JMC 76, JMC 8 and JMC 58 produced a similar amount of biomass with reduced metabolic cost of soil exploration compared to the cultivar JMC 57. In the case of low P-tolerant soybean cultivars: GmJMC033, GmJMC040 and GmJMC085, GmJMC085 showed remarkable responses with regards to RDW, TRL and SRL compared to the other two cultivars under low P conditions. Thus, soybean cultivar GmJMC085 produced greater biomass with reduced metabolic cost of soil exploration than soybean cultivar GmJMC033. Besides, genotypes with reduced metabolic costs of soil exploration are imperative to improve P acquisition under low P stress. In addition, the maize cultivars JMC 13, JMC 71, and soybean, the cultivars GmJMC064, GmJMC059 and GmJMC106 were P-inefficient due to poor modification in root growth. Our results highlighted that compared to low P-sensitive genotypes

of both species, low P-tolerant genotypes could modify their root system by increasing RRS, TRL, and SRL to acquire P to cope with P deficiency.

P accumulation and PUE:

Shoot P concentrations were significantly lower in low P treatment in maize and soybean cultivars except for GmJMC059 and GmJMC106. The notable genotypic variation in shoot P concentrations could be found under both species' high P but not the low P. The shoot and root P concentrations were lower in P-tolerant cultivars than in P-sensitive cultivars, e.g., in maize cultivars JMC 57 and JMC 71 and the soybean cultivars GmJMC085 and GmJMC106. Therefore, the genotypes with poor biomass and high tissue P concentrations were considered poor performers under low P conditions. However, P deprivation caused a significant increase in the PUE in both maize and soybeans. The notable genotypic variation in PUE could be found in maize and soybean cultivars under low P, indicating that some cultivars efficiently utilized acquired P more than others under low P conditions.

ACP and rhizosphere acidification:

The amelioration in ACP activity of rhizosphere soil of maize under low P condition seemed distinct in all cultivars. However, it was not prominent in P-sensitive soybean cultivars: GmJMC064, GmJMC059 and GmJMC106 under low P. It further indicates that ACP activity under low P depends on plant species, and compared to maize, soybean resulted in weak ACP activity in rhizosphere soil. The soybean cultivars GmJMC040, GmJMC085 and GmJMC033 are among the cultivars that showed higher ACP activity under low P, indicating that low P-tolerant cultivars of soybean are characterized by high ACP activity in rhizosphere soil. These variations are imperative for future breeding ventures in producing P-efficient genotypes.

Compared to maize cultivars, the reduction in rhizosphere pH was noticeable among the soybean cultivars under low P. Among the low P-tolerant maize cultivars, only JMC 76 significantly reduced rhizosphere soil pH and the low P-tolerant soybean cultivars, GmJMC033 and GmJMC085, significantly dropped down the rhizosphere soil pH under low P conditions However, our results highlighted that maize cultivars showed low P tolerance; JMC 76, JMC 57, JMC 58 and JMC 8 highly depended on

root morphological traits rather than physiological traits. Further, the soybean cultivar GmJMC085, characterized by well-defined morphological and physiological responses under P deficiency, indicated that low P tolerance was due to both responses in soybean.

Contrasting responses of maize and soybean under low P stress:

Maize seems more responsive to P under high P conditions than soybeans. It drastically reduced the shoot P growth under low P stress than soybean reduced. Both species were characterized by high RRS, sustained TRL and SRL under P deficiency. In contrast, maize had significantly higher TRL and SRL than soybean, indicating that contrasting root traits evolved in efficient P acquisition. The possible reasons for the higher TRL and SRL in maize would be aerenchyma formation, root hair proliferation and enhanced or sustained lateral rooting to maximize soil exploration at minimum metabolic cost. However, the high P uptake efficiency of soybeans is supported by the fineness of the root system. Under low P stress, both species significantly improved the PUE, whereas soybean resulted in higher PUE than maize under both P conditions. Furthermore, maize enhanced the ACP activity notably more than soybean under P-impoverished conditions, which can facilitate mobilizing sparingly available P in soils. Therefore, significant variation among species and genotypes in the same species exists regarding the root traits under low P stress. Hence, further exploiting genotypic variation is needed for better crop performance under low P stress.

Conclusions:

We observed significant genotypic variation in selected cultivars of Japanese core collections of maize and soybean under low and sufficient P conditions. The results showed that the plasticity of the root system characterized by high RRS, SRL and TRL contributes to the differences among genotypes. Their distinct modifications in morphological and physiological traits are crucial in considering future breeding ventures to produce more P-efficient crop genotypes. Among the tested maize cultivars, JMC 76, JMC 57, JMC 58 and JMC 8 and under the tested soybean cultivars GmJMC033, GmJMC040, and GmJMC085 showed distinctive root modification under low P stress, showing their low P tolerance. Further, identifying their quantitative trait loci (QTLs)

would be more beneficial in understanding the genetic basis for their adaptations under low P stress. These genotypic adaptations could be combined with agronomic strategies to enhance the overall P use efficiency in diversified cropping systems like intercropping.

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